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<b>(54) Title:</b> HETERODUPLEX TRACKING ASSAY (HTA) FOR GENOTYPING HCV  <b>(57) Abstract</b>  A heteroduplex tracking assay (HTA), a hybridization based method of determining the genetic relationship between two or more viral genomes, for genotyping HCV is disclosed. The HTA for genotyping HCV was developed using single stranded probes derived from the carboxyl terminus of core and part of the E1 for HCV subtypes (1a, 1b, 2a, 2b, and 3a). HTA is more accurate than RFLP for sub-typing HCV and has potential for identifying new variants and is useful for epidemiological studies.		

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## HETERODUPLEX TRACKING ASSAY (HTA) FOR GENOTYPING HCV

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### Field of the Invention

This invention relates to genotyping hepatitis C viruses (HCV). In particular, this invention relates to specific primers preferably from the core and envelope region of HCV and a method to determine genotypes of HCV with a heteroduplex mobility or tracking assay which, in turn, utilizes specific primers.

### Background of the Invention

Viral hepatitis is known to be caused by five different viruses known as hepatitis A, B, C, D, and E. HAV is an RNA virus and does not lead to long-term clinical symptoms. HBV is a DNA virus. HDV is a dependent virus that is unable to infect cells in the absence of HBV. HEV is a water-borne virus. HCV was first identified and characterized as a cause of non-A, non-B hepatitis NANBH. (Houghton et al., EPO Pub. Nos. 388,232 and 318,216). This led to the disclosure of a number of general and specific polypeptides useful as immunological reagents in identifying HCV. See, e.g., Choo et al. (1989) Science, 244:359-262; Kuo et al., (1989) Science 244:362-364 and Houghton et al, (1991) Hepatology 14:381-388.

HCV is a single stranded RNA virus, distantly related to the pestivirus and flavivirus and it is the causative agent of the vast majority of transfusion-associated hepatitis and of most cases of community-acquired non-A, non-B hepatitis worldwide. The HCV genome consists of 5' and 3' noncoding (NC) regions that flank a single long open reading frame (ORF). This ORF encodes for three structural proteins at the amino-terminal end and for six nonstructural (NS) proteins at the carboxyl-terminal end. The structural proteins are represented from the nucleocapsid (core; C) proteins and two glycoproteins, envelope 1 (E1) and envelope 2 (E2). The nonstructural proteins are named NS2, NS3, NS4a, NS4b, NS5a, NS5b. The 5'NCR is the most highly conserved part of the HCV genome, whereas the sequence of the two envelope proteins (E1 and E2) is highly variable among different HCV isolates. The highest degree of variation has been observed in a region within E2, now commonly termed hypervariable region 1

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(HVR1) or E2HV. A second variable region called the HVR2 also exists in a subset of isolates. Typically, the genetic heterogeneity of HCV has been classified under two headings quasispecies and genotypes. As used herein the term "quasispecies" refers to the genetic heterogeneity of the HCV population within an infected individual. As used  
5 herein the terms "genotype" and "subtype" refer to the genome heterogeneity observed among different HCV isolates. The analysis of nucleic acid sequence variation of the HCV genome, a positive stranded of approximately 9.4 kb RNA molecule, suggest that genetic variability is associated with important virological and clinical implications.

The prototype isolate of HCV was characterized in EP Publications Nos. 318,216  
10 and 388,232. As used herein, the term "HCV" includes newly isolated NANBH viral species. The term "HCV-1" refers to the virus described in the above-mentioned publications.

Since the initial identification of HCV, at least 6 different major viral types have been identified (full length genomes reported) and designated Type 1, 2, 3, 4, 5 and 6.  
15 Within these types are numerous subtypes. The type of virus with which a patient is infected may affect the clinical prognosis and also response to various treatments. See, Yoshioke et al., (1992) Hepatology 16:293-299. Considering that the most serious clinical outcome of HCV infection is hepatocellular carcinoma, it would be useful to be able to determine with which type or types of HCV a patient is infected. It is thus of  
20 particular importance to develop an, accurate, reliable assay for HCV genotyping and subtyping, that, without requiring the sequencing, could also give the genetic divergence intra-subtype. Several classification have been proposed for HCV genotyping based on analysis of different regions, because the ideal nucleotide sequence-based system, using the complete viral genome is not practical.

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#### Summary of the Invention

The present invention includes primers and methods for the characterization of HCV genotyping and of variation intra-subtype based on the heteroduplex tracking assay (HTA). The preferred probes/primers were single stranded derived from the carboxyl  
30 terminus of core and part of the E1 region of HCV.

The HTA is a hybridization based method of determining the genetic relationship between two or more viral genomes. The basis of the method is that related DNA products coamplified from divergent templates reanneal randomly to form heteroduplexes that migrate with reduced mobility in systems designed to separate molecules on the basis of size such as neutral polyacrylamide gels, HTA was originally used to genotype HIV-1 and to follow the in vivo evolution of HIV-1 in patients and populations. See, e.g., Delwart et al., (1993) Science 262:1757-1261 and Delwart et al., (1994) J. Virol. 68:6772-6883.

One aspect of the invention is a method for genotyping HCV comprising the steps of denaturing and reannealing partially complementary DNA or RNA strands and detecting sequence variation by noting electrophoretic mobility of the DNA heteroduplexes on a system designed to separate molecule on the basis of size such as by following electrophoresis through a polyacrylamide or MDE gel.

Another aspect of the invention relates to the probes used in the genotyping which were selected from the core and E1 region of the HCV genome.

Another aspect of the invention relates to a method of predicting the response to drug therapy of a patient infected with a strain of HCV by determining the sensitivity of different known genotypes to drug therapy, determining the genotype of the HCV strain infecting the patient and comparing the genotype with its drug therapy sensitivity to predict the patient's response to the drug therapy.

Another aspect of the invention relates to therapeutic vaccines and predicting which therapeutic vaccine should be utilized by determining the genotype of a patient infected with a strain of HCV and administering a therapeutic vaccine of the same genotype.

Another aspect of the invention relates to prophylactic vaccines and predicting which vaccine should be administered to a certain population sample by determining the prevalent genotypes in a like sample and administering a prophylactic vaccine of a genotype likely to be the prevalent genotype to the population sample.

Another aspect of the invention relates to the ability to discovering new genotypes of HCV using the method of the invention.

### Brief Description of the Figures

Figures 1A - 1E are autoradiograms showing homoduplexes and heteroduplexes of the samples to be typed with the probes of known genotypes (ss probes are of genotypes 1a, 1b, 2a, 2b, 3a in Figs. 1A- 1E respectively, lane on far left of MDE gel). The homoduplex (h) (ss probe to the double stranded RT-PCR product of known genotypetpfrom which it was derived) is shown adjacent to the probe. The heteroduplexes of the RT-PCR products from the 15 dialysis patients (nos. 1, 2, 3, 4, 7, 18, 20, 22, 23, 24, 26, 28, 30, 33, 35) hybridized to the ss probe is designated above the appropriate lane in each Figure.

Figures 2A - 2C are dendograms, i.e., phylogenetic trees showing the relatedness of each partial E1 nucleotide sequence,, formed by comparing partial E1 sequences obtained by sequencing of putative type 1 (nt 625-930), type 2 (nt 583-915) or type 3 (nt 558-834) isolates from the dialysis patients described hereinto published genotype sequences for type 1a (HCV-1) (Choo, et al, PNAS (1991) 88:2451-2455, all nucleotide, "nt", designations according to this paper), 1b (HCV-J) (Kato et al, PNAS (1990) 87:9524-2528), 2a (HC-J6) (Okamoto et al Virol. (1992) 188:331-341), 2b (HC-J8) (Okamoto et al Virol. (1992) 188:331-341), 2c (Bukh, et al PNAS (1993) 90:8234-8239 ) and 3a (NZL-1) (Sakamoto, et al) J. Gen. Virol. (1994) 75:1761-1768 over the same region of the genome.

Figure 2D is a dendogram, phylogenetic tree, formed by comparing either partial 5'UTR sequences of isolates 23, 30 and 33 obtained by direct sequencingwith published type 1, 2 and 3 (nt -274 to -81) genotype sequences for the same region of the genome.

Figures 3A - 3D show the nucleotide sequences for dendograms depicted in Figures 2A - 2D.

### Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, polypeptide and nucleic acid synthesis, and immunology, which are within the skill of

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- the art. Such techniques are explained fully in the literature. See e.g., Sambrook, et al.,  
MOLECULAR CLONING; A LABORATORY MANUAL, SECOND EDITION  
(1989); DNA CLONING, VOLUMES I AND II (D.N Glover ed. 1985);  
OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed, 1984); NUCLEIC ACID  
5 HYBRIDIZATION (B.D. Hames & S.J. Higgins eds. 1984); TRANSCRIPTION AND  
TRANSLATION (B.D. Hames & S.J. Higgins eds. 1984); ANIMAL CELL CULTURE  
(R.I. Freshney ed. 1986); IMMOBILIZED CELLS AND ENZYMES (IRL Press, 1986);  
B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984); the series,  
METHODS IN ENZYMOLOGY (Academic Press, Inc.); GENE TRANSFER  
10 VECTORS FOR MAMMALIAN CELLS (J.H. Miller and M.P. Calos eds. 1987, Cold  
Spring Harbor Laboratory), Methods in Enzymology Vol. 154 and Vol. 155 (Wu and  
Grossman, and Wu, eds., respectively), Mayer and Walker, eds. (1987),  
IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY  
(Academic Press, London), Scopes, (1987), PROTEIN PURIFICATION: PRINCIPLES  
15 AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF  
EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D.M. Weir and C. C. Blackwell  
eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this  
specification. All publications, patents, and patent applications cited herein are  
20 incorporated by reference.

The term "recombinant polynucleotide" as used herein intends a polynucleotide  
of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or  
manipulation:

- (1) is not associated with all or a portion of a polynucleotide with which it is  
25 associated in nature, (2) is linked to a polynucleotide other than that to which it is linked  
in nature, or (3) does not occur in nature.

The term "polynucleotide" as used herein refers to a polymeric form of  
nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term  
refers only to the primary structure of the molecule. Thus, this term includes double- and  
30 single-stranded DNA and RNA. It also includes known types of modifications, for

example, labels which are known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

By "PCR" is meant herein the polymerase chain reaction (PCR) technique, disclosed by Mullis in U.S. Pat. Nos. 4,683,195 (Mullis et al) and 4,683,202, incorporated herein by reference. In the PCR technique, short oligonucleotide primers are prepared which match opposite ends of a desired sequence. The sequence between the primers need not be known. A sample of DNA (or RNA) is extracted and denatured (preferably by heat). Then, oligonucleotide primers are added in molar excess, along with dNTPs and a polymerase (preferably Taq polymerase, which is stable to heat). The DNA is replicated, then again denatured. This results in two "long products," which begin with the respective primers, and the two original strands (per duplex DNA molecule). The reaction mixture is then returned to polymerizing conditions (e.g., by lowering the temperature, inactivating a denaturing agent, or adding more polymerase), and a second cycle initiated. The second cycle provides the two original strands, the two long products from cycle 1, two new long products (replicated from the original strands), and two "short products" replicated from the long products. The short products have the sequence of the target sequence (sense or antisense) with a primer at each end. On each additional cycle, an additional two long products are produced, and a number of short products equal to the number of long and short products remaining at the end of the previous cycle. Thus, the number of short products grows exponentially with each cycle. This amplification of a specific analyte sequence allows the detection of extremely small quantities of DNA.

The term "3SR" as used herein refers to a method of target nucleic acid amplification also known as the "self-sustained-sequence-replication" system as described in European Patent Publication No. 373,960 (published June 20, 1990).

5 The term "LCR" as used herein refers to a method of target nucleic acid amplification also known as the "ligase chain reaction" as described by Barany, Proc. Natl. Acad. Sci. (USA) (1991) 88:189-193.

An "open reading frame" (ORF) is a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

10 A "coding sequence" is a polynucleotide sequence which is translated into a polypeptide, usually via mRNA, when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, cDNA, and recombinant polynucleotide sequences.

15 As used herein, the term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to or exclude post expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example,  
20 polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

A polypeptide or amino acid sequence "derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a  
25 polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least 3-5 amino acids, and more preferably at least 8-10 amino acids, and even more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence. This terminology also includes a polypeptide expressed from a designated nucleic acid sequence.

The protein may be used for producing antibodies, either monoclonal or polyclonal, specific to the protein. The methods for producing these antibodies are known in the art.

"Recombinant host cells", "host cells," "cells," "cell cultures," and other such terms denote, for example, microorganisms, insect cells, and mammalian cells, that can be, or  
5 have been, used as recipients for recombinant vector or other transfer DNA, and include the progeny of the original cell which has been transformed. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation. Examples for mammalian host cells include Chinese hamster ovary  
10 (CHO) and monkey kidney (COS) cells.

By "cDNA" is meant a complimentary mRNA sequence that hybridizes to a complimentary strand of mRNA.

By "purified" and "isolated" is meant, when referring to a polypeptide or nucleotide sequence, that the indicated molecule is present in the substantial absence of other  
15 biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000, can be present).

20 By "pharmaceutical acceptable carrier," is meant any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers; and inactive virus particles. Such carriers are well known to  
25 those of ordinary skill in the art.

The therapeutic compositions typically will contain pharmaceutically acceptable vehicles, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect.

5 Evidence indicates that different HCV genotypes may have different pathogenicities as well as distinct geographical distributions and may elicit partly different serological profiles in infected patients. See Cammarota, et al. J. Clin. Microb. (1995) 33:2781-2784. The invention includes methods for detecting HCV and identifying infection by different types of HCV. The invention includes genotyping  
10 HCV, the potential to discover a new genotype of HCV, and assessing viral populations for ability to predict response to drug therapy. The invention also includes probes for use in the genotyping of HCV.

The methods for genotyping HCV include but are not limited to a heteroduplex tracking or mobility assay utilizing probes/primers from the core/E1 region of the HCV  
15 genome. The documented antigenic differences between HCV genotypes would have usefulness not only in blood donor screening and in predicting response to IFN treatment, but also for the designated composition of candidate vaccines for HCV in different countries, choice of therapeutic vaccines, as well as in the identification of new genotypes. Other methods have been proposed to identify the main genotypes infecting  
20 populations, based on analysis of different regions of the genome, such as RFLP. See Davidson et al., J. Gen Virol. (1995) 76:1197-1204 for discussion of genotyping HCV using RFLP of sequences amplified from the 5' non-coding region (NCR).

The known nucleic acid based methods of genotyping require a sub-type specific RT-PCR(reverse transcriptase-PCR) primers (see Okamoto (1992) J. Gen Virol 73:673-  
25 679) U.S. Patent 5,427,909; (2) specific probes (G. Marteen, et al., Line probe assay); (3) restriction site polymorphism (a function of the nucleotide sequence (nt)) or (4) direct sequences to determine genotype. The analysis of the 5' NC sequence with RFLP is easy to perform, but does not accurately predict all HCV genotypes, and, some subtypes may  
30 be misclassified. For example, the change in sequence between 1a and 1b recognized by the restriction enzyme is not absolute and sequences other than 1a and 1b, and 2a and 2b

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are misclassified. For example, type 1c would appear as type 1a, type 2c as either type 2a or 2b. See Cammarota et al., J. Clin. Microb., (1995) 33:2781-2784. For this reason, RFLP is not able to detect "escape" species, new divergent species, or epidemiological trends. It is likely that a typing method like RFLP will have to be continuously modified to accommodate the rapidly increasing information collected on HCV sequence heterogeneity.

As above-mentioned, when using the nucleic acid based methods of genotyping, one obtains a result of either a type or subtype or a negative that is "untypeable" result. See, e.g. Cammarota, et al., J. Clin. Microb. (1995) 33:2781-2784, isolates that remained untyped by genotype-specific PCR were classified subtype 2c on the basis of sequence analysis of PCR amplications obtained from the core and NS5 genes. This problem is avoided by using the presently claimed invention to determine HCV genotypes by choosing RT-PCR primers in the C-terminus or core/mid 2/3 of E1. In addition, the subtype of the isolate can be accurately determined using the present invention of HCV genotyping and isolates can be detected, even those less than in approximately 30% divergent, enabling the characterization of new sub-types without sequencing.

#### Heteroduplex Tracking or Mobility Assay

The method of determining the genotype of HCV in the present invention utilizes minor variants in complex quasispecies. One such technique is the heteroduplex tracking assay (HTA). HTA, well known in the art for use with HIV, (see e.g., Delwart, et al., J. Virol. (1994) 68:6672-6683; Delwart, et al., Science (1993) 262:1257-1261; Delwart, et al., PCR Methods and Applications 4:S202-S216 (1995 Cold Springs Harbor; and Delwart, et al., Heteroduplex Mobility Analysis HIV-1 env Subtyping Kit Protocol Version 3, each of which is incorporated herein by reference in its entirety), grew out of the observation that when sequences were amplified by nested PCR from peripheral blood mononuclear cells of infected individuals, related DNA products coamplified from divergent templates could randomly reanneal to form heteroduplexes that migrate with reduced mobility in neutral polyacrylamide gels. Using these techniques, one can establish genetic relationships between multiple viral DNA template molecules.

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HTA in particular utilizes a first PCR product as a labeled probe, it may be radioactive, which is mixed with an excess (driver) of an unlabeled PCR product from a different source, i.e., the source for which typing is desired. The probe sequences are then driven completely into heteroduplexes with the driver, and are separated on the basis of size. An autoradiogram for example of the resulting polyacrylamide gel reveals only these heteroduplexes and provides a visual display of the relationship between the two virus populations under study. The fact that heteroduplexes migrate with distinct mobilities indicates that the strand-specific composition of mismatched and unpaired nucleotides affects their mobility.

An exponential equation described in Delwart et al., is then used to describe a curve fitting the experimental data from pairwise analysis of genes of known sequence. In the present invention, the equation is used to estimate the genetic distance between the known genotypes of the probes and the unknown genotypes of the patient samples.

#### 15 Primers for Use in the HTA

It was determined that the E1 or core region could be the best region in to study the HCV heterogeneity, thus the E1 region became the choice for primers in the present invention. The use of the partial E1 sequence, the most heterogeneous region of the genome for the present invention, as well as a longer fragment, i.e. 400nt, although it could have been as long as 1000nt, enabled the design of probes which do not cross hybridize among sub-types/types and thus allow accurate geneotyping. By flanking the heterogeneous region, conserved nt sequences for sense and antisense primers were identified. Preferably, a combination of universal sense and type specific antisense primers for the first PCR round and a universal antisense and type specific sense primers for the second round were utilized. The PCR need not be two rounds and the primers are not limited to the above-described combination. The preferred combination, however, enabled the preparation of single stranded probes and minimized the number of PCR primer combinations.

Preferred probes are sequences in the core and E1 regions of which the sequences for a wide range of genotypes are published and grouped into at least 12

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distinct genotypes and subtype: I/1a, II/1b, III/2a, IV/2b, 2c, 3a, 4a, 4b, 4c, 4d, 5a, 6a.

The nucleotide sequence identities of the E1 gene among HCV isolates of the same genotype ranges from 88.0% to 99.1%, whereas those of HCV isolates of different genotypes ranges from 53.5 to 78.6%. The degree of variation for good discrimination of heteroduplex in neutral polyacrylamide gels is comfortably within the range of 3-20%, so that is likely that divergent templates reanneal to form a heteroduplex if they are of the same subtype. For this reason, a single stranded 32p labelled DNA probe was used so that if the formation of the heteroduplex is impossible, the ss-DNA probe could likely not reanneal and form a homoduplex band. Without direct sequencing, the present invention can rapidly give not only a certain identification of the subtypes, but also the genetic relations inside the same subtypes. For example, the genotypes analyzed, i.e., (1a, 1b, 2a, 2b, 3a) showed no overlapping between different subtypes.

Further since isolates approximately 30% divergent can be visualized on the gel- new subtypes can be visualized and the distribution of isolates in a population could be characterized and populations or individual isolates can be followed in population or in individuals in epidemiological studies.

#### HCV Genotyping Kits

A kit for determining the genotype of HCV is within the scope of this invention.

As described for HIV in Delwart et al, Heteroduplex Mobility Analysis HIV-1 env Subtyping Kit Protocol Version 3, such a kit would include the specific primers.

Preferred primers are from the core and E1 region of the HCV genome. If two stages of PCR are desired, the first round primers could include for example a universal sense probe, preferably located in the core/E1 region of the HCV genome. One such universal primer is located from nucleotide 508 to 529 of HCV-1 and is shown in Table 1.

Coupled with the universal primer could be a type specific antisense primer also preferably located in the core/E1 region of the HCV genome. Examples of these primers are from nucleotides 1032 to 1012 for type 1, type 2a, type 2b and type 3a of the HCV genomes and are also shown in Table 1.

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If a second round of PCR is desired, the second round primers would likewise be from the core/E1 region of the HCV genome. Preferred second round primers could include a universal antisense primer from nucleotides 978 to 958 of the HCV-1 genome, this primer is shown in Table 1. In addition the second round primers could include a type specific sense primer from the core/E1 region. Preferred second round type specific sense primers are from nucleotides 536 to 557 of HCV genomes type 1, type 2 or type 3, and are shown in Table 1.

The first or second round of primers may be sufficient to amplify the viral RNA without using a second round of PCR if the concentration of the virus is sufficiently high, ie., nested PCR is not necessarily required, what is required is PCR products in 100x excess of probe.

An HCV genotyping kit of the present invention would also include subtype references which may change as new subtypes are discovered and evaluated for use in the kit. Use of more than one reference from a given subtype is recommended because comparison to a single reference does not always provide an unambiguous result.

The foregoing discussion and following examples only illustrate the invention, persons of ordinary skill in the art will appreciate that the invention can be implemented in other ways, and the invention is defined solely by reference to the claims.

### Example 1

#### Patient samples

35 hemodialyzed patients undergoing regular hemodialysis were studied: 20 men (57%) and 15 women (43%) with a mean age of  $64.8 \pm 13$  years. Serum samples were collected in August 1995, divided into aliquots and stored at -80 degrees Celsius. 26 patients were anti-HCV ELISA positive and 9 anti-HCV ELISA negative. 25 of the 26 ELISA positive were also RIBA III positive, while 1 was indeterminate. The 9 ELISA negative were all RIBA III negative. 15 patients were HCV-RNA 5' UTR and E1 PCR positive. By direct sequencing of 15 5' NCR products, 5 patients resulted type 1; 3 patients type 2; and 7 patients type 3.

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Example 2cDNA and PCR

HCV-RNA was extracted at least two different times using a Stratagene reagent from a Stratagene RNA Isolation Kit (Chomezynsky and Sacchi method).

5 RNA extracted from 20ul of plasma that was reverse transcribed in a 25 ul of cDNA mixture (BRL cDNA synthesis kit, 8085SB) using 100 pmol of PCR primers. The cDNA mixture was boiled for 5 minutes, quick-cooled on ice and added to the PCR cDNA reagents with final concentrations according to the Perkin PCR kit (N801-0055) specification. 40 PCR cycles (94 degrees Celsius for 10 seconds, 55 degrees Celsius for  
10 30 seconds and 72 degrees Celsius for 30 seconds were performed. Ten ul of the first PCR reaction mixture was added to a second PCR reaction mixture containing nested PCR primers and was amplified for 40 cycles as indicated above.

The first extraction was used for the nested-PCR reaction with primers specific for the 5' NCR as previously described in Shimizu et al, PNAS (1992) 5477-5481 and  
15 this product was directly sequenced and used for the RFLP. RNA from the same extraction was used for HTA using core/E1 primers. A second RNA extraction was performed for RFLP and/or HTA to confirm the results. The primers used for the HTA are listed in Table 1. The nested pairs of PCR primers used to obtain these E1 products were different for the types 1, 2a, 2b, and 3a. The universal sense probe for the first  
20 round of amplification corresponds to 5'-3' nt 508-529, amino acids 170-176, of Choo, et al., PNAS, 1991, while the universal antisense primer for the second round of amplification corresponds to nt 978-958, amino acids 320-326 of Choo, et al., PNAS, 1991.

When the ssDNA DNA probes were prepared for use in the HTA, one of the  
25 primers for the nested PCR was biotinylated. See e.g. SEQ ID NO:6 in Table 1.

15  
Table 1

	HCV-1	5' → 3' nt	3' → 5' nt	~ Amino Acid	Primer Type
5	(SEQ ID NO:1) Purified, C170S	508-529	529-508	170-176	Universal sense probe PCR I
10	(SEQ ID NO:2) Purified, E338A1	1032-1012	1012-1032	338-344	Type 1 antisense PCR I
	(SEQ ID NO:3) Purified, E338A2a	1032-1012	1012-1032	338-344	Type 2a antisense PCR I
15	(SEQ ID NO:4) Purified, E338A2b	1032-1012	1012-1032	338-344	Type 2b antisense PCR I
	(SEQ ID NO:5) Purified, E338A3a	1032-1012	1012-1032	338-344	Type 3a antisense PCR I
20	(SEQ ID NO:6) Purified, E320A	978-958	958-978	320-326	Universal antisense PCR II
25	(SEQ ID NO:7) Purified, C179S1	536-557	958-978	179-186	Type 1 sense PCR II
	(SEQ ID NO:8) Purified, C179S2	536-557	958-978	179-186	Type 2 sense PCR II
30	(SEQ ID NO:9) Purified, C179S3	536-557	958-978	179-186	Type 3 sense PCR II

### Example 3

35

#### HTA

The single stranded probes were prepared by RT-PCR of HCV ELISA and RIBA positive sera of known genotypes with the same PCR primers described, as above, except that one of the primers 320A was biotinylated. ssDNA probes were generated with the Dynabeads M-280 Streptavidin following the protocol of Heng Pan and Eric Delwart.

40

The non-biotinyl single strand was eluted from the magnetic bead/streptavidin column. Probes were generated from 20 ng of ssDNA of the different genotypes and end labeled using T4 polynucleotide kinase (Gibco BRL) and 100 microCi of 32P ATP and then column purified. The kinase probe was separated from 32P ATP using a Pharmacia

45

Bio Sepharose column. The 32P-labeled single strand probes were mixed with a 100-fold excess driver, and the PCR products were generated from the patient samples or the control serum/plasma. Hybridization was in 2 x SSC. The mixtures were put on a 94 degree Celsius heat block for 3 minutes. They were then transferred to a 55 degree

16

Celsius heat block for at least 2 hours. The entire reaction volume was loaded on 1mm thick, 6% polyacrylamide MDE gel (Baker) and electrophoresed for 16 h at 500V. The gel was vacuum dried at 80 degrees Celsius on filter paper and exposed to X-ray film. The genotypes of each of the samples were determined based on the Delwart method.

5 Table 2 depicts the genotype results determined by using HTA.

Figures 1A-1E are autoradiograms showing each of the single strand probes in Table 1, that is the probes specific known for genotypes 1a, 1b, 2a, 2b, 3a in Figures 1A-E respectively, see the lane on the far left of the MDE gel. The homoduplex(h) (ss probe to the double stranded RT-PCR product form which it was derived) is shown adjacent to the probe. RT-PCR products from the 15 dialysis patients (nos. 1, 2, 3, 4, 7, 18, 20, 22, 10 23, 24, 26, 28, 30, 33, 35) hybridized to the probe is designated also as the appropriate lane in each Figure.

As can be seen in Figures 1A-1E, Type 1 ss subtypes probes were specific for each type 1 sub-type and did not cross hybridize with other subtypes 1b, 2a, 2b, 3a (2a, 15 2b not shown). Type 3a ss sub-type specific probe was also specific for subtype 3a and did not cross hybridized with 1a, 2c, or 2a, 2s isolates (data not shown). ss Sub-type 2 probes do not cross hybridize with each other (data not shown) but did cross-hybridized with subtype 2c isolates; however, the distance between the homoduplex and the 2c isolates indicates a high degree of divergence suggesting that patients 23, 30 and 33 had 20 different sub-types. The virus in sera 23, 30 and 33 was confirmed by sequencing the partial E1 to be most closely related to sub-type 2c (see figure 2b) but was ambiguous by 51UTR sequencing, See Figure 2D.

Isolates 23, 30 and 33 hybridized with the 2a probe, while only 30 and 33 hybridized to the 2b probe. The gels also indicate that isolate 30 is more closely related 25 to 2a than to 2b. Therefore, while all three sera are clearly type 2 non-a, non-b subtype, they are not all equally divergent from types 2a and 2b. As seen in Figures 1B and 1D, patient 4 appears to be co-infected with types 1b and a non-a, non-b type subtype.

The 1b probe was derived from a patient (JK 16) and appeared to have two viral genomes which is reflected in the homoduplex lane (h) and therefore each 1b patient has 30 two bands.

The ss probe 3a was derived from a plasmid clone of one RT-PLR product from a type 3a individual (JK3a), see Fig. 1E, lane h, therefore, multiple bands in lane 22 most likely reflect two closely related viruses in this patient.

5 It appeared that most often patients had unique viral isolates. It is possible that patents 3 and 18 had identical or highly related virus isolates. Similarly, patients 20 and 26 had the same type 3a viral isolate and patients 2 and 4 has the same type 1b isolate based on the co-migration of the bands on MDE gels.

10 Figures 2a-2c depict phylogenetic trees, dendrograms, showing the genetic relatedness of each of the partial E1 nucleotide sequences. These denrograms were constructed by pairwise progressive alignment of the nucleotide sequences to one another by using the computer software program GeneWorks Unweighted Pair Group Methods with Arithmetic mean, as described in Weiner, et al., *J. Virol.* 67: pg. 4365-4368 (1993). The dendrograms, in Figures 2a-2c were formed by comparing partial E1 sequences of putative type 1 (nt 625-93), type 2 (nt 583-915) or type 3 (nt 558-834) isolates from the  
15 dialysis patients, as determined by sequence analysys to published genotype sequences for type 1a (HCV-1) (Choo, et al. PNAS 1991); 1b (HCV-J) (Kato et al.); 2a (HC-J6)(Okamoto, et al (1992); 2b (HC-J8)(Okamoto, et al, 1992); 2c (Bukh, et al.PNAS 1993) and 3a (NZL-1) (Sakamoto, et al. 1994) over the same region of the genome.

20 Figure 2D is a dendrogram formed as above-described by comparing either partial 5' UTR sequences of isolates 23, 30 and 33 with published type 1, 2 and 3 (nt-274 to -81) genotype sequences for the same region of the genome.

The results of the RFLP and HTA were compared and are presented in Table 2.

18  
Table 2

Comparison of Partial E1 HTA and RFLP Genotyping Results

5	Patient	HTA	RFLP
10	1	1b	1b
	2	1b	1b
	3	3a	3a
	4	1b	1b
	7	3a	3a
	18	3a	3a
	20	3a	3a
15	22	3a	3a
	23	2?*	2a
	24	1b	1b
	26	3a	3a
	28	1b	1b
20	30	2?*	2a
	33	2?*	2a
	35	3a	3a
25	* sample is neither 2a nor 2b		

The partial E1 sequences depicted in Figures 3a-3d confirm the HTA sub-type designations given in Table 2 and definitively show that patients 23, 30 and 33 are most closely related to 2c with 33 being the most distantly related to 2c. (18.6% divergent).

30 The RFLP results using ScrFI (see Davidson, et al., J. Gen. Virol. (1995) 76:1197-1204) wrongly designated 23, 30 and 33 as type 2a. This wrong designation is reflected in Figure 2D which shows that based on the 5' UTR nt sequence, the computer did not accurately sub-type HCV 2c due to insufficient nt divergence in this region of the genome.

35 The present invention of HTA utilizing primers for the core and envelope region allowed for 3 levels of characterization of HCV genomes. The first was type specificity in the choice of RT-PCR primers. The second was sub-type specificity, based on choosing primers in the core/E1 region, and from a region greater than 400 nt, which

19  
resulted in a lack of cross-hybridization between sub-type probes, e.g. 1 and 3, 2a, 2b;  
and a high degree of heterogeneity to maximize differences between genotypes (lack of  
cross-hybridization). Finally, isolate specificity was determined by the distance from the  
homoduplex as exemplified in Figures 1.E - 1-E. Other genotyping methods do not have  
5 the ability to analyze isolate differences

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: CHIRON CORPORATION
- (ii) TITLE OF INVENTION: HETERODUPLEX TRACKING ASSAY (HTA) FOR GENOTYPING HCV
- (iii) NUMBER OF SEQUENCES: 52
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Chiron Corporation
  - (B) STREET: 4560 Horton Street - R440
  - (C) CITY: Emeryville
  - (D) STATE: California
  - (E) COUNTRY: USA
  - (F) ZIP: 94608-2916
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: Unassigned
  - (B) FILING DATE: Even date herewith
  - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Harbin, Alisa A.
  - (B) REGISTRATION NUMBER: 33,895
  - (C) REFERENCE/DOCKET NUMBER: 1226.100
- (ix) TELECOMMUNICATION INFORMATION:
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  - (C) TELEX: N/A

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTGGTTGCT CTTTCTCTAT CT

22

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs

21

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

---

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATGGCTTGT GGGATCCGGA G

21

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATGACCTCG GGGACGCGCA T

21

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GACCAGTTCT GGAACACGAG C

21

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAAGGTCTGG GGTAAACGCA G

21

---

(2) INFORMATION FOR SEQ ID NO:6:

---

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

22

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCAGTTCATC ATCATATCCC A

21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGCCCTGCT CTCTTGCTTG AC

22

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTGCTCTTCT GTCGTGCGTC AC

22

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTGCTCTGTT CTCTTGCTTA AT

22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

23

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACTCAAGCA TTGTGTATGA AGCGGCGGAC ATGATCATGC ACACCCCCGG GTGCGTGCCA	60
TGCGTCCGGG AGGGCAATCT CTCCCGCTGC TGGGTAGCGC TCACTCCAC GCTCGCGGCC	120
AGAAACAGCA GCGTTCCTAC TACGACAATA CGACGCCATG TCGACTTGCT AGTAGGAGCG	180
GCTGCTTTT GCTCCGCCAT GTACGTGGGG GACCTCTGCG GATCTATTTT CCTCGTCTCC	240
CAACTGTTCA CTTTCTCGCC CGCCGGCAT CATAAGTAC AGGACTGCAA TTGCTCGATC	300
TATCCC	306

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AACTCAAGCA TCGTGTATGA GGCAGCGGAA GTGATCATGC ACATTCCCCG GTGCGTGCCC	60
TGCGTTCGGG AGAGCAATCT CTCCCGCTGC TGGGTAGCGC TCACCCAC ACTCGCGGCC	120
AGGAACAGCA GCGTCCCCAC CACGACAATA CGACGCCACG TCGACTTGCT CGTTGGGGCG	180
GCTGCCTTCT GCTCCGCTAT GTATGTGGGG GATCTCTGCG GATCTGTTT CCTTGTCTCC	240
CAACTGTTCA CTTTTCGCC TCGCCGGCAT GAGACAGTAC AGGACTGCAA TTGTTCAATC	300
TATCCC	306

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AACTCAAGCA TAGTATATGA GGCAGCGGAA ATAATCATGC ATACCCCCGG GTGCGTGCCC	60
TGTGTTCCGG AGGTCAATC CTCCCGCTGC TGGGCAGCGC TCACCCCTAC GCTCGCGGCC	120
AGGAACTCCA GCGTGCCAC TACGACAATA CGACGCCACG TCGACTTGCT CGTTGGGGCG	180

GCTGCTTTCT GCTCCGCTAT GTACGTGGGG GATCTATGCG GATCTGTTCT ACTTGTCTCT 240  
CAGCTGTTCA CTTTCTCACC TCGCCGGCAC GAGACAGTGC AGGACTGCAA TTGTTCAATC 300  
TATCCC 306

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 306 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AACACGAGCA TTGTGTATGA GGCAGCGGAC TTGATCATGC ACGTCCCCGG GTGCGTGCCC 60  
TGCGTTCGGG AGGGCAACTC CTCCCGATGC TGGGTAGCGC TCACTCCAC GATCGCGGCC 120  
AGGAACAGCA GTGTCCCGT TACGACCATA CGACGCCACG TCGATTGCT CGTTGGGGCG 180  
GCTGCTCTTT GCTCCGCCAT GTACGTGGGG GATCTCTGCG GATCTGTCTT CCTCGCTTCC 240  
CAGTTGTTCA CTTTCTCGCC TCGCCAGCAT CAGACGGTAC AGGACTGCAA CTGCTCAATC 300  
TATCCC 306

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 306 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AACTCAAGCA TCGTGTATGA GGCGGCGGAA GTGATCATGC ACATTCCTGG GTGCGTGCCC 60  
TGCGTTCGGG AGGGCGACTT CTCCCGCTGC TGGGTAGCGC TCACCCAC ACTCGCGGCC 120  
AGGAATAACA GCGTCCCCAC TACGACAATA CGACGCCACG TCGACTTGCT CGTTGGGGCG 180  
GCTGCCTTCT GCTCCGCTAT GTACGTGGGG GATCTCTGCG GATCTGTTTT CTTGTCTCC 240  
CAACTGTTCA CTTTTTCGCC TCGCCGGCAT GCGACAGTAC AGGACTGCAA TTGTTCAATC 300  
TATCCC 306

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 306 base pairs

25

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
AACTCGAGTA TTGTGTACGA GCGGCCGAT GCCATCCTGC AACTCCGGG GTGCGTCCCT      60
TGC GTTCGTG AGGGCAACGC CTCGAGGTGT TGGGTGGCGA TGACCCCTAC GGTGGCCACC      120
AGGGATGGCA AACTCCCCGC GACGCAGCTT CGACGTCACA TCGATCTGCT TGTCGGGAGC      180
GCCACCCTCT GTTCGGCCCT CTACGTGGGG GACCTATGCG GGTCTGTCTT TCTTGTCGGC      240
CAACTGTTCA CTTTCTCTCC CAGGCGCCAC TGGACGACGC AAGGTTGCAA TTGCTCTATC      300
TATCCC                                           306
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 306 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
AACTCAAGTA TTGTGTATGA GGCAGCGGAC ATGATCATGC ACACCCCGG GTGCGTGCCC      60
TGC GTCCGGG AGAGTAATTT CTCCCGTTGC TGGGTAGCGC TCACTCCAC GCTCGCGGCC      120
AGGAACAGCA GCATCCCCAC CACGACAATA CGACGCCACG TCGATTTGCT CGTTGGGGCG      180
GCTGCTCTCT GTTCGCTAT GTACGTTGGG GATCTCTGCG GATCCGTTTT TCTCGTCTCC      240
CAGCTGTTCA CTTTCTCACC TCGCCGGTAT GAGACGGTAC AAGATTGCAA TTGCTCAATC      300
TATCCC                                           306
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 306 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
AATGATAGCA TTACCTGGCA ACTCCAGGCT GCTGTCCTCC ACGTCCCCGG GTGCGTCCCC      60
```

TGCGAGAAAG TGGGGAATAC ATCTCGGTGC TGGATACCGG TCTCACCGAA TGTGGCOGTG	120
CAGCAGCCCG GCGCCCTCAC GCAGGGCTTA CGGACGCACA TTGACATGGT TGTGATGTCC	180
GCCACGCTCT GCTCCGCTCT TTACGTGGGG GACCTCTGCG GTGGGGTGAT GCTTCAGCC	240
CAGATGTTCA TTGTCTCGCC ACAGCACCAC TGGTTTGTGC AAGACTGCAA TTGCTCCATC	300
TACCCT	306

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AACAACAGCA TCACCTGGCA GCTCACTGAC GCACTTCTCC ATCTTCCTGG ATGCGTCCCA	60
TGTGAGAATG ATAATGGCAC CTTGCATTGC TGGATACAAG TAACACCCAA CGTGGCTGTG	120
AAACACCGCG GTGCGCTCAC TCGTAGCCTG CGAACACACG TCGACATGAT CGTAATGGCA	180
GCTACGGCCT GCTCGGCCTT GTATGTGGGA GATGTGTGCG GGGCCGTGAT GATTCTATCG	240
CAGGCTTTCA TGGTATCACC ACAACGCCAC AACTTCACCC AAGAGTGCAA CTGTTCCATC	300
TACCAA	306

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AATAGCAGTA TTGTGTATGA GGCCGATGAT GTCATTCTGC ACACACCCGG CTGTGTACCT	60
TGTGTCCAGG ACGGCAATAC ATCTACGTGC TGGACCCAG' TGACACCTAC AGTGGCAGTC	120
AGGTACGTCG GAGCAACTAC TGCTTCGATA CGCAGTCATG TGGACCTATT AGTAGGCGCG	180
GCCACGATGT GCTCTGCGCT CTACGTGGGT GATATGTGTG GGGCTGTCTT TCTCGTGGGA	240
CAAGCCTTCA CGTTCAGACC TCGACGCCAT CAAACGGTCC AGACCTGTAA CTGCTCGCTG	300
TACCCA	306

## (2) INFORMATION FOR SEQ ID NO:20:

27

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs  
(B) ~~TYPE: nucleic acid~~  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
CGCAACTCCA CGGGGCTTTA CCACGTCACC AATGATTGCC CTAACGAG TATTGTGTAC      60
GAGACGGCCG ATGCCATCCT GCACACTCCG GGGTGGCTCC CTTGTGTTTCG CGAGGGCAAC    120
GCCTCGAGGT GTTGGGTGGC GATGACCCCT ACGGTGGCCA CCAGGGATGG CAAACTCCCC    180
GCGACGCAGC TTCGACGTCA CATCGATCTG CTTGTGGGA GCGCCACCCT CTGTTGCGCC    240
CTCTACGTGG GGGATCTGTG CGGGTCTGTC TTTCTGTGCG GCCAACTGTT TACCTTCTCT    300
CCCAGGCGCC ACTGGACGAC GCAAGGTTGC AAT                                     333
```

## (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
AAGAACACCA GCGACAGCTA CATGGTGACC AATGACTGCC AAAATGACAG CATCACCTGG      60
CAGCTTGAGG CTGCGGTCCT CCACGTCCCC GGGTGGCTCC CGTGCGAGAG AGTGGGAAAT    120
ACATCTCGGT GCTGGATACC GGTCTACCA AACGTGGCTG TCGGCAGCC CGGCGCCCTC    180
ACGCAGGGCT TCGGACGCA CATCGACATG ATTGTGATGT CCGCCACGCT CTGCTCCGCT    240
CTCTACGTGG GGGACCTCTG TGGCGGGATG ATGCTCGCAG CCCAGATGTT CATCGTTTCG    300
CCGCAGAACC ACTGGTTCGT GCAGGAATGC AAT                                     333
```

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) ~~LENGTH: 333 base pairs~~  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

28

AGGAACATCA GTTCTAGCTA CTACGCCACT AATGACTGCT CGAACAACAG CATCACCTGG 60  
 CAGCTCACCA ACGCAGTTCT CCACCTTCCC GGATGCGTCC CATGTGAGAA TAATAATGGC 120  
 ACCTTGCAAT GCTGGATACA AGTAACACCT AATGTGGCCG TAAAACATCG CGGCGCACTC 180  
 ACTACAACC TCGGACACA TGTCGACATG ATCGTAATGG CAGCTACGGT CTGTTGGGCC 240  
 TTGTACGTAG GAGACGTGTG TGGGGCTGTG ATGATTGTGT CTCAGGCCCT TATAATATCA 300  
 CCAGAACACC ATAACCTCAC CCAAGAGTGC AAC 333

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AAGGACACCG GCGACTCCTA CATGCCGACC AACGATTGCT CCAACTCTAG TATCGTTTGG 60  
 CAGCTTGAAG GAGCAGTGCT TCATACTCCT GGATGCGTCC CTTGTGAGCG TACCGCCAAC 120  
 GTCTCTCGAT GTTGGGTGCC GGTGCCCCC AATCTCGCCA TAAGTCAACC TGGCGCTCTC 180  
 ACTAAGGGCC TCGAGCACA CATCGATATC ATCGTGATGT CTGCTACGGT CTGTTCTGCC 240  
 CTTTATGTGG GGGACGTGTG TGGCGCGCTG ATGCTGGCCG CTCAGGTCGT CGTCGTGTGC 300  
 CCACAACACC ATACGTTTGT CCAGGAATGC AAC 333

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AAAAACACCA GCATCTCCTA TATGGCGACC AACGACTGCT CCAATTCCAG CATCGCTTGG 60  
 CAGTTTGACG GCGCAGTGCT CCATACTCCT GGATGTGTCC CTTGCGAACG GACCGGCAAC 120  
 GCGTCCCGGT GTTGGGTGCC GGTGCCCCC AATGTGGCTA TAAGACAACC CGGCGCCCTC 180  
 ACTAAGGGCA TACGAACGCA CATTGATGTC ATCGTAATGT CTGCTACGCT CTGTTCTGCC 240  
 CTTTACGTGG GGGACGTGTG TGGTGCGCTG ATGATTGCCG CTCAGGTCGT CATTGTGTCT 300

CCGCAGCATC ACCACTTTGT CCAGGACTGC AAT

333

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 333 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAGAACACCA GCGACTCCTA CATGGCGACT AACGACTGCT CTAAGTCCAG CATCGTTTGG	60
CAGCTTGAGG ACGCAGTGCT CCATGTCCCT GGATGTGTCC CTTGTGAGAA GACTGGCAAT	120
ACGTCTCGGT GCTGGGTGCC GGTTACCCCC AATGTGGCTA CAAGTCAACC CGGCGCTCTC	180
ACCAGGGGCT TGGGACGCA CATCGATGTC ATCGTGATGT CAGCCACGCT CTGCTCCGCT	240
CTCTATGTGG GGGACGTGTG TGGCGCGTTG ACGATAGCCG CTCAGGTTGT CATCGTATCG	300
CCACGGCACC ACCACTTTGT CCAGGACTGC AAT	333

## (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 333 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAGAACACCA GCACCTCCTA CATGGTGACT AACGATTGCT CCAACTCCAG CATCGTTTGG	60
CAACTTGAAG GCGCAGTGCT CCATGTTTCCT GGATGTGTCC CTTGTGAGCA GATCGGCAAC	120
GTGTCTCAGT GTTGGGTGCC GGTTACCCCC AATATGGCCA TAAGTACACC CGGCGCTCTC	180
ACTAAGGGCT TGCGAACGCA CATCGACGGC ATCGTGATGT CCGCTACGCT CTGTTCTGCC	240
CTTTATGTGG GGGACGTGTG TGGCGCGTTG ATGATAGCCG CCCAGGTCGT CATCGTATCG	300
CCACAGCACC ACCACTTTGT CCACGACTGC AAC	333

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 333 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGCAACTCCA CGGGGCTTTA CCACGTCACC AATGATTGCC CTAACGAG TATTGTGTAC	60
GAGGCGGCCG ATGCCATCCT GCACACTCCG GGGTGCCTCC CTTGCGTTCG TGAGGGCAAC	120
GCCTCGAGGT GTTGGGTGGC GATGACCCCT ACGGTGGCCA CCAGGGATGG CAAACTCCCC	180
GCGACGCAGC TTCGACGTCA CATCGATCTG CTTGTGCGGA GCGCCACCCT CTGTTGCGCC	240
CTCTACGTGG GGGACCTATG CGGGTCTGTC TTTCTGTGCG GCCAACTGTT CACCTTCTCT	300
CCCAGGCGCC ACTGGACGAC GCAAGGTTGC AAT	333

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGCAACGTGT CCGGGATATA CCATGTCACG AACGACTGCT CCAACTCAAG TATTGTGTAT	60
GAGGCAGCGG ACATGATCAT GCACACCCCC GGGTGCCTGC CCTGCGTCCG GGAGAGTAAT	120
TTCTCCCGTT GCTGGGTAGC GCTCACTCCC ACGCTCGCGG CCAGGAACAG CAGCATCCCC	180
ACCACGACAA TACGACGCCA CGTCGATTG CTCGTTGGGG CGGCTGCTCT CTGTTCCGCT	240
ATGTACGTTG GGGATCTCTG CGGATCCGTT TTTCTCGTCT CCCAGCTGTT CACCTTCTCA	300
CCTCGCCGGT ATGAGACGGT ACAAGATTGC AAT	333

## (2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGGAACATTA GTTCTAGCTA CTACGCCACT AATGATTGCT CAAACAACAG CATCACCTGG	60
CAGCTCACTG ACGCAGTTCT CCATCTTCCT GGATGCGTCC CATGTGAGAA TGATAATGGC	120
ACCTTGCACT GCTGGATACA AGTAACACCC AACGTGGCTG TGAAACACCG CGGTGCGCTC	180
ACTCGTAGCC TGCGAACACA CGTCGACATG ATCGTAATGG CAGCTACGGC CTGCTCGGCC	240

3/

TTGTATGTGG GAGATGTGTG CGGGGCCGTG ATGATTCTAT CGCAGGCTTT CATGGTATCA 300  
CCACAACGCC ACAACTTCAC CCAAGAGTGC AAC 333

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## (2) INFORMATION FOR SEQ ID NO:30:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CGGAATACGT CTGGCCTCTA CGTCCTTACC AACGACTGTT CCAATAGCAG TATTGTGTAT 60  
GAGGCCGATG ATGTCATTCT GCACACACCC GGCTGTGTAC CTTGTGTCCA GGACGGCAAT 120  
ACATCTACGT GCTGGACCCC AGTGACACCT ACAGTGGCAG TCAGGTACGT CGGAGCAACT 180  
ACTGCTTCGA TACGCAGTCA TGTGGACCTA TTAGTAGGCG CGGCCACGAT GTGCTCTGCG 240  
CTCTACGTGG GTGATATGTG TGGGGCTGTC TTTCTCGTGG GACAAGCCTT CACGTTTACA 300  
CCTCGACGCC ATCAAACGGT CCAGACCTGT AAC 333

## (2) INFORMATION FOR SEQ ID NO:31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAGAACATCA GTACCGGCTA CATGGTGACC AACGACTGCA CCAATGATAG CATTACCTGG 60  
CAACTCCAGG CTGCTGTCCT CCACGTCCCC GGGTGCCTCC CGTGCGAGAA AGTGGGGAAT 120  
ACATCTCGGT GCTGGATACC GGTCTCACCG AATGTGGCCG TGCAGCAGCC CGGCGCCCTC 180  
ACGCAGGGCT TACGGACGCA CATTGACATG GTTGTGATGT CCGCCACGCT CTGCTCCGCT 240  
CTTTACGTGG GGGACCTCTG CGGTGGGGTG ATGCTTGCAG CCCAGATGTT CATTGTCTCG 300  
CCACAGCACC ACTGGTTTGT GCAAGACTGC AAT 333

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## (2) INFORMATION FOR SEQ ID NO:32:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACATTATTG TGTATGAGGC CGATGACGTC ATCCTGCACA CGCCCGGCTG	120
TGTACCTTGT GTTCAGGACG GTAATACATC CAAGTGCTGG ACCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGTCGGAG CAACCACCGC TTCAATACGC AGCCACGTGG ACCTATTATT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACATCATTG TGTATGAGGC CGATGACGTC ATCCTGCACG CACCCGGCTG	120
TGTACCTTGT GTTCAGGACG GCAATACATC CACGTGCTGG ACCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGTCGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTATTAGT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AATATTATTG TGTATGAGGC CGACGACGTC ATCCTGCACG CCCCCGGCTG	120
TGTACCTTGT GTTCAGGACG GCAATACATC CACGTGCTGG ATCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGCCGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTGTTAGT	240

GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT

33

277

## (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 277 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACATTATTG TGTATGAGGC CGATGACGTC ATCCTGCACA CACCCGGCTG	120
TGTACCTTGT GTTCAGGACG GCAATACATC CACGTGCTGG ACCCCAGTGA CACCTACAGT	180
ATCAGTCAGG TACGTCGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTACTATT	240
GGGCGCGGCC ACGATGTGCT CCGCGCTCTA CGTGGGT	277

## (2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 277 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACAGTATTG TGTATGAGGC CGATCAGTC ATCCTGCACA CACCCGGCTG	120
TGTACCTTGT GTTCAAGCCA ACAATAAATC CAAATGCTGG ACCCCAGTGA CACCTACAGT	180
ATCAGTCGAG TACGTCGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTACTATT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

## (2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 277 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

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TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA 60  
CTGTTCTAAT AACATTATTG TGTATGAGGC CGATGACGTC ATCCTGCACA CACCCGGCTG 120  
TGTACCTTGT GTTCAGGACG GCAATGCATC CACGTGCTGG ACCCCAGTAA CACCTACAGT 180  
ATCAGTCAGG TACGTCGGAG CAACCACCGC TTCAGTACGC AGCCATGTGG ACCTACTATT 240  
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA TGTGGGT 277

## (2) INFORMATION FOR SEQ ID NO:38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TCACCAACGA 60  
CTGTTCCAAC AACATTATTG TGTATGAGGC CGATGACGTC ATTCTGCACA CGCCCGGCTG 120  
CGTACCTTGT GTACAGGACG GCAATACATC CACGTGCTGG ACCCCAGTGA CACCTACAGT 180  
GGCAGTCAGG TACGTCGGAG CAACTACCGC TTCAATACGC AGCCATGTGG ACCTATTATT 240  
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT 277

## (2) INFORMATION FOR SEQ ID NO:39:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TCATCCAGCA GCCAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTACGTCC TTACCAACGA 60  
CTGTTCCAAT AGCAGTATTG TGTATGAGGC CGATGATGTC ATTCTGCACA CACCCGGCTG 120  
TGTACCTTGT GTCCAGGACG GCAATACATC TACGTGCTGG ACCCCAGTGA CACCTACAGT 180  
GGCAGTCAGG TACGTCGGAG CAACTACTGC TTCGATACGC AGTCATGTGG ACCTATTAGT 240  
AGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT 277

## (2) INFORMATION FOR SEQ ID NO:40:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid

3.5

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

~~(ii) MOLECULE TYPE: DNA (genomic)~~

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGTGCCCCGCT TCGGCCTACC AAGTGCGCAA CTCCACGGGG CTTTACCACG TCACCAATGA	60
TTGCCCTAAC TCGAGTATTG TGTACGAGGC GGCCGATGCC ATCCTGCACA CTCCGGGGTG	120
CGTCCCTTGC GTTCGTGAGG GCAACGCCTC GAGGTGTTGG GTGGCGATGA CCCCTACGGT	180
GGCCACCAGG GATGGCAAAC TCCCCGCGAC GCAGCTTCGA CGTCACATCG ATCTGCTTGT	240
CGGGAGCGCC ACCCTCTGTT CGGCCCTCTA CGTGGGG	277

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 277 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CATCCCAGCT TCCGCTTACG AGGTGCGCAA CGTGTCCGGG ATATACCATG TCACGAACGA	60
CTGCTCCAAC TCAAGTATTG TGTATGAGGC AGCGGACATG ATCATGCACA CCCCCGGGTG	120
CGTGCCCTGC GTCCGGGAGA GTAATTTCTC CCGTTGCTGG GTAGCGCTCA CTCCCACGCT	180
CGCGGCCAGG AACAGCAGCA TCCCCACCAC GACAATACGA CGCCACGTCG ATTTGCTCGT	240
TGGGGCGGCT GCTCTCTGTT CCGCTATGTA CGTTGGG	277

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 277 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

~~(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:~~

CACCCCGGTC TCCGCTGCCG AAGTGAAGAA CATCAGTACC GGCTACATGG TGACCAACGA	60
CTGCACCAAT GATAGCATTG CCTGGCAACT CCAGGCTGCT GTCCTCCACG TCCCCGGGTG	120
CGTCCCGTGC GAGAAAGTGG GGAATACATC TCGGTGCTGG ATACCGGTCT CACCGAATGT	180
GGCCGTGCAG CAGCCCGGCG CCCTCAGCA GGGCTTACGG ACGCACATTG ACATGGTTGT	240

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GATGTCCGCC ACGCTCTGCT CCGCTCTTTA CGTGGGG

277

## (2) INFORMATION FOR SEQ ID NO:43:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

AGTGCCAGTG TCTGCAGTGG AAGTCAGGAA CATTAGTTCT AGCTACTACG CCACTAATGA	60
TTGCTCAAAC AACAGCATCA CCTGGCAGCT CACTGACGCA GTTCTCCATC TTCCTGGATG	120
CGTCCCATGT GAGAATGATA ATGGCACCTT GCATTGCTGG ATACAAGTAA CACCCAACGT	180
GGCTGTGAAA CACCGCGGTG CGCTCACTCG TAGCCTGCCA ACACACGTCG ACATGATCGT	240
AATGGCAGCT ACGGCCTGCT CGGCCTTGTA TGTGGGA	277

## (2) INFORMATION FOR SEQ ID NO:44:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGTA CAGCCTCCAG GCCCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAGACT	120
GGGTCCTTTC TTGATAAAC CCACTCTATG CCCGGTCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

## (2) INFORMATION FOR SEQ ID NO:45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGTA CAGCCTCCAG GCCCCCCCCT	60
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CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTAC CGGAAAGACT 120

GGGTCCTTTC TTGGATAAAC CCACTCTATG TCCGGTCATT TGGGCACGCC CCCGCAAGAC 180

TGCTAGCCGA GTAG 194

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCTGT CAGCCTCCAG GACCCCCCT 60

CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CAGGACGACC 120

GGGTCCTTTC TTGGATCAAC CCGCTCAATG CCTGGAGATT TGGGCGTGCC CCCGCGAGAC 180

TGCTAGCCGA GTAG 194

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCTGT CAGCCTCCAG GACCCCCCT 60

CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CAGGACGACC 120

GGGTCCTTTC TTGGATCAAC CCGCTCAATG CCTGGAGATT TGGGCGTGCC CCCGCAAGAC 180

TGCTAGCCGA GTAG 194

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

38

GCGGAAAGCG CCTAGCCATG GCGTTAGTAC GAGTGTCTGTG CAGCCTCCAG GACCCCCCCT 60  
CCCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATCGC TGGGGTGACC 120  
GGGTCCTTTC TTGGAGCAAC CCGCTCAATA CCCAGAAATT TGGGCGTGCC CCCGCGAGAT 180  
CACTAGCCGA GTAG 194

## (2) INFORMATION FOR SEQ ID NO:49:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCTGTG CAGCCTCCAG GACCCCCCCT 60  
CCCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAGACT 120  
GGGTCCTTTC TTGGATAAAC CCACTCTATG CCCGGCCATT TGGGCGTGCC CCCGCAAGAC 180  
TGCTAGCCGA GTAG 194

## (2) INFORMATION FOR SEQ ID NO:50:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCTGTG CAGCCTCCAG GACCCCCCCT 60  
CCCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAGACT 120  
GGGTCCTTTC TTGGATAAAC CCACTCTATG CCCGGCCATT TGGGCGTGCC CCCGCAAGAC 180  
TGCTAGCCGA GTAG 194

## (2) INFORMATION FOR SEQ ID NO:51:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGCGTA CAGCCTCCAG GCCCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CAGGAAGACT	120
GGGTCCTTTC TTGGATAAAC CCACTCTATG CCGTGGCCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

## (2) INFORMATION FOR SEQ ID NO:52:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGCGTA CAGCCTCCAG GTCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAGACT	120
GGGTCCTTTC TTGGATAAAC CCACTCTATG CCGGCGCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

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What is claimed is:

1. An oligonucleotide consisting of the sequence of Seq ID No. 1.
2. An oligonucleotide consisting of the sequence of Seq ID No. 2.
3. An oligonucleotide consisting of the sequence of Seq ID No. 3.
4. An oligonucleotide consisting of the sequence of Seq ID No. 4.
5. An oligonucleotide consisting of the sequence of Seq ID No. 5.
6. An oligonucleotide consisting of the sequence of Seq ID No. 6.
7. An oligonucleotide consisting of the sequence of Seq ID No. 7.
8. An oligonucleotide consisting of the sequence of Seq ID No. 8.
9. An oligonucleotide consisting of the sequence of Seq ID No. 9.
10. A pair of PCR primers wherein the sense primer consists of Seq ID NO. 1 and the antisense primer is selected from the group consisting of Seq ID NO 2, Seq ID NO. 3, Seq ID NO. 4 and Seq ID NO. 5.
11. A pair of PCR primers wherein the antisense primer consists of Seq ID NO. 6 and the sense primer is selected from the group consisting of Seq ID NO 7, Seq ID NO. 8, and Seq ID NO. 9.
12. A method of determining the HCV genotype of an HCV strain, said method comprising the steps of:
  - (a) subjecting said HCV strain to one or more stages of PCR, wherein the one or more stages of PCR utilizes a sense probe from the core or E1 region of the HCV genome and an antisense probe from the core or E1 region of the HCV genome;
  - (b) forming a heteroduplex by denaturing and reannealing mixtures of the amplified product obtained in step (a) with DNA or RNA fragments of a known HCV genotype;

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(c) comparing the mobility of said heteroduplex on a system that separates by size with the mobility of a homoduplex of the DNA or RNA fragments of known genotype to determine the genotype of the HCV strain.

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13. The method of claim 12 wherein said HCV strain is subjected to two stages of PCR, wherein the first set of primers comprise a universal sense probe from the core or E1 regions of the HCV genome and a type specific antisense probe from the core or E1 regions of the HCV genome, and wherein the second set of PCR primers comprise a universal antisense probe from the core or E1 regions of the HCV genome and a type specific sense probe from the core or E1 regions of the HCV genome.

14. The method of claim 12 wherein the first set of PCR primers are those according to claim 10 and wherein the second set of PCR primers are those according to claim 11.

15. The method of claim 12 wherein said DNA or RNA fragments of a known genotype comprise a DNA probe.

16. The method of claim 15 wherein said probe is single stranded.

17. The method of claim 16 wherein said DNA probe is radiolabeled.

18. The method of claim 16 wherein said single stranded DNA probe is obtained by PCR amplification.

19. The method of claim 18 wherein said DNA probe is obtained by two step PCR amplification utilizing the primers of claim 10 for the first step and claim 11 for the second step.

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20. The method of claim 12 wherein said HCV strain is present in an excess in the mixture forming the heteroduplex.

21. A method to predict the response to drug therapy of a strain of HCV from a patient infected with said strain of HCV, said method comprising determining the sensitivity of known HCV genotypes to said drug therapy, determining the HCV genotype of said strain of HCV by the method according to claim 12, and comparing said HCV genotype of said strain prior to said drug therapy with said sensitivity of known HCV genotypes to said drug therapy.

22. A method to predict the response to a therapeutic vaccine of a strain of HCV from a patient infected with said strain of HCV, said method comprising determining the sensitivity of known HCV genotypes to said therapeutic vaccine, determining the HCV genotype of said strain of HCV by the method according to claim 12, and comparing said HCV genotype of said strain prior to administration of said therapeutic vaccine with said sensitivity of known HCV genotypes to said therapeutic vaccine.

23. A method to predict the appropriateness of a prophylatic vaccine composition for a given sample population said method comprising determining the genotype of said prophylatic vaccine, determining the predominance of known HCV genotypes in said sample population by the method according to claim 12, and comparing said HCV genotype of said prophylatic vaccine strain to the determined predominant genotype prior to administration of said prophylatic vaccine to said population sample.

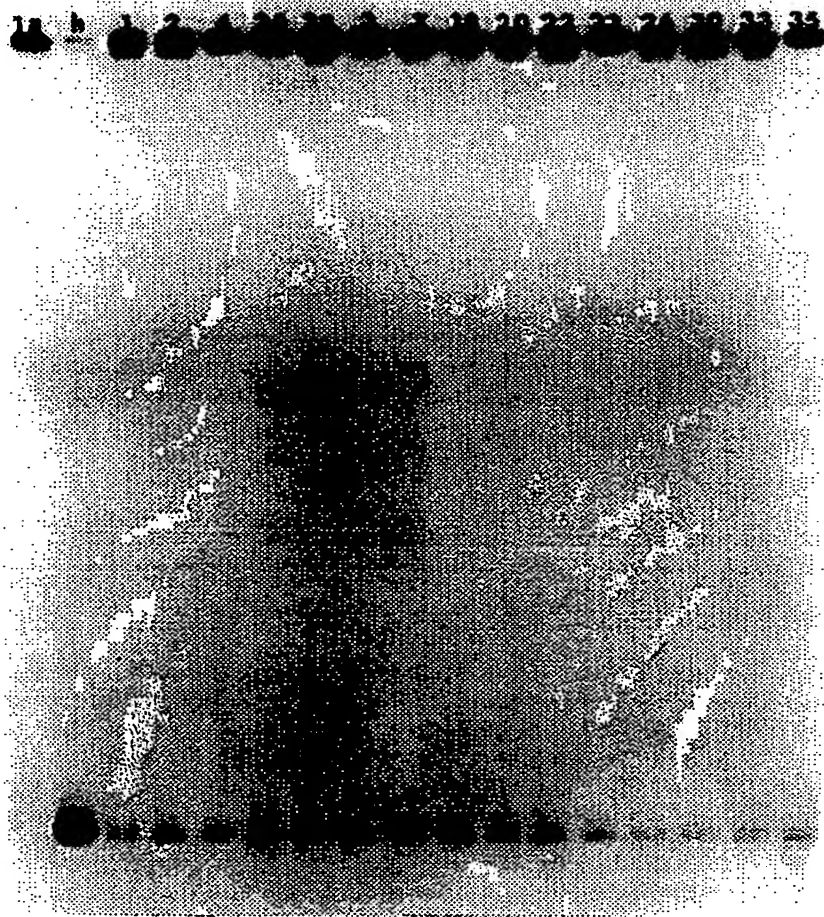


FIG. 1A

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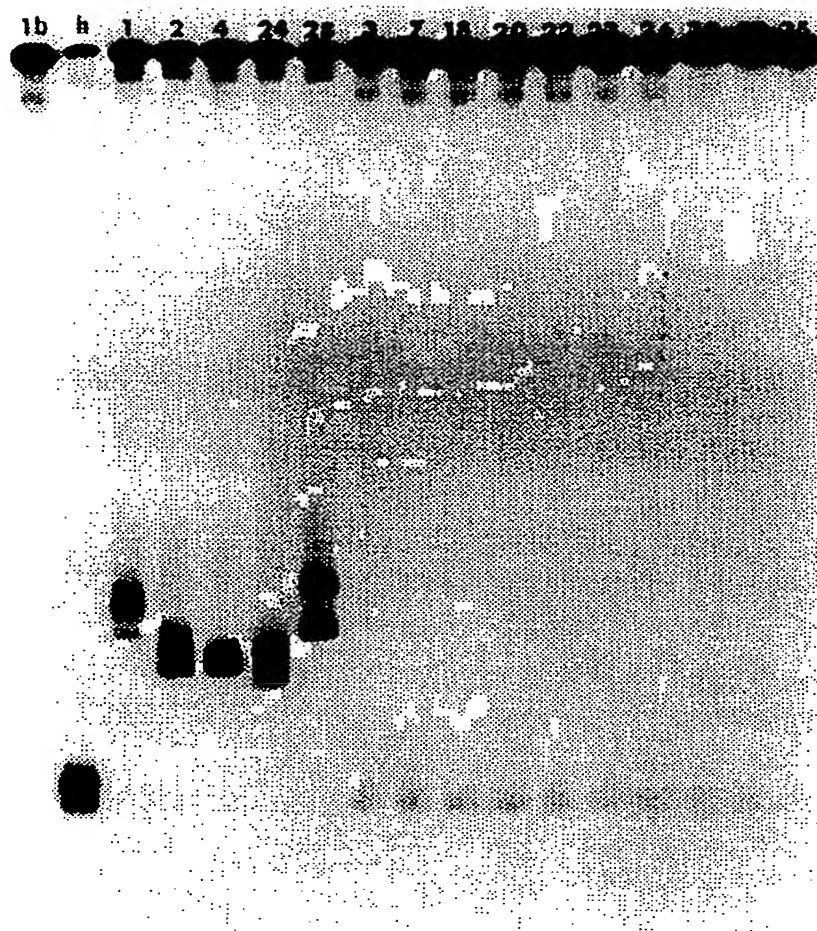


FIG. 1B

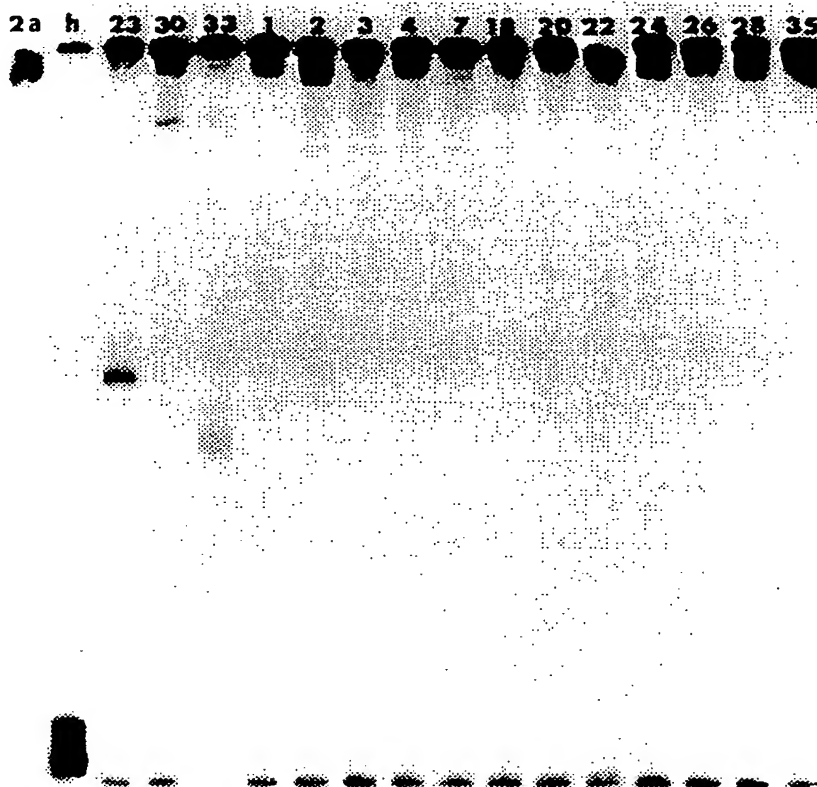


FIG. 1C

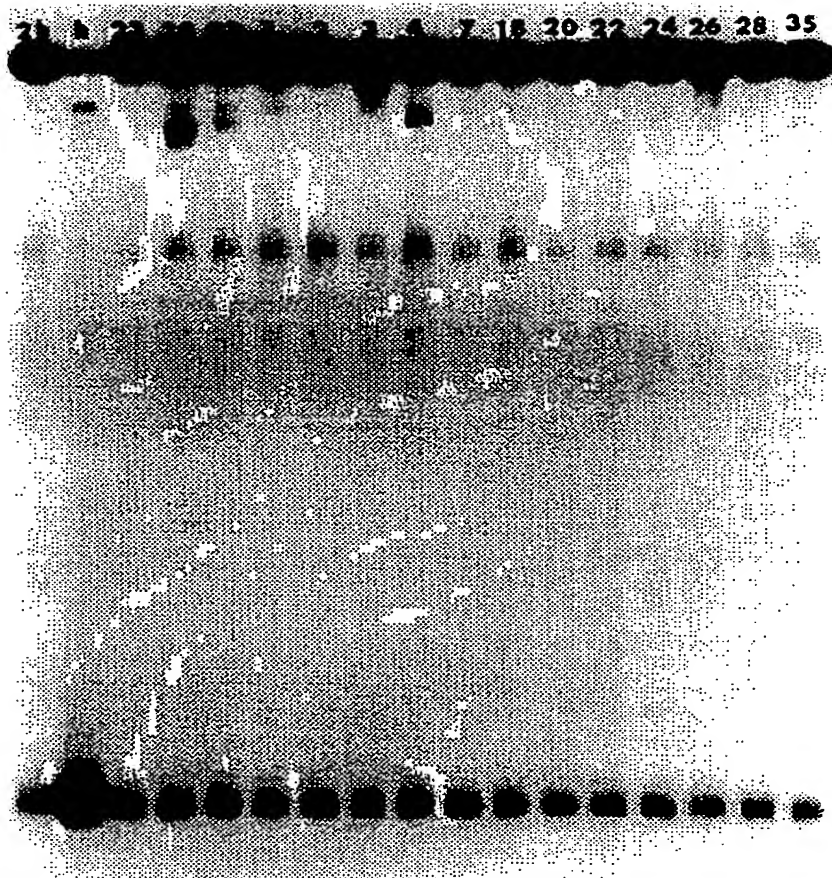


FIG. 1D

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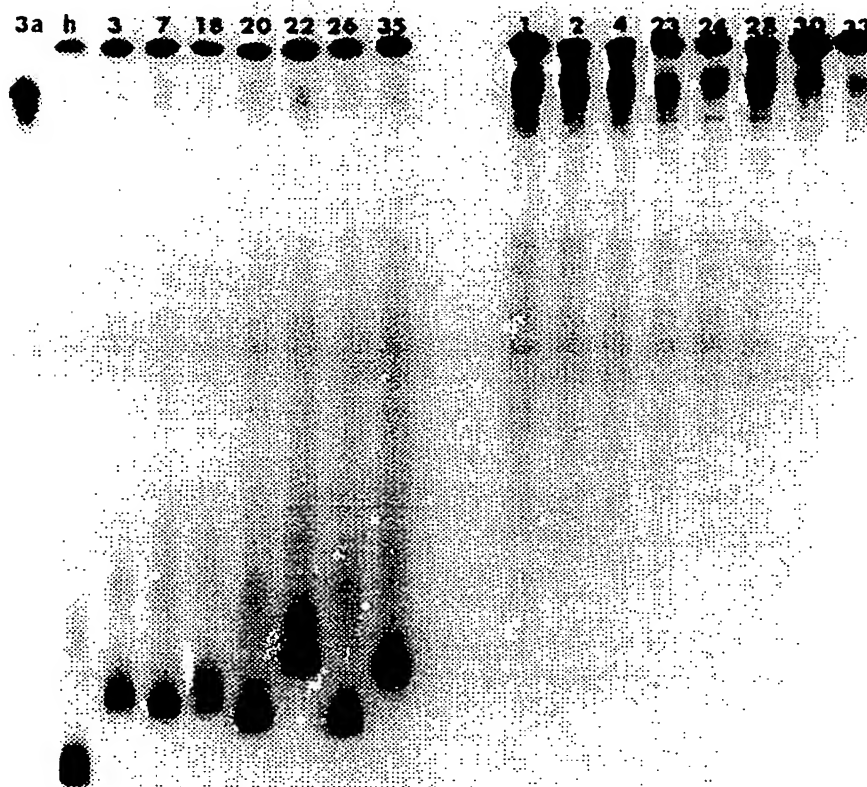


FIG. 1E

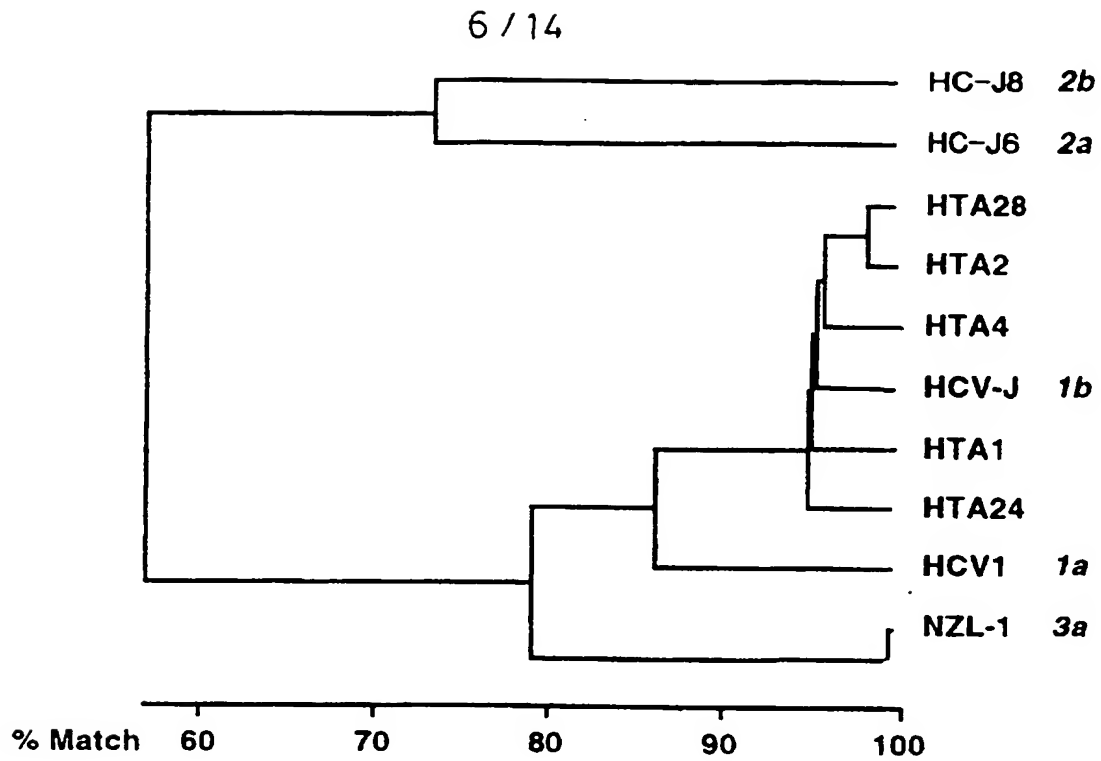


FIG. 2A

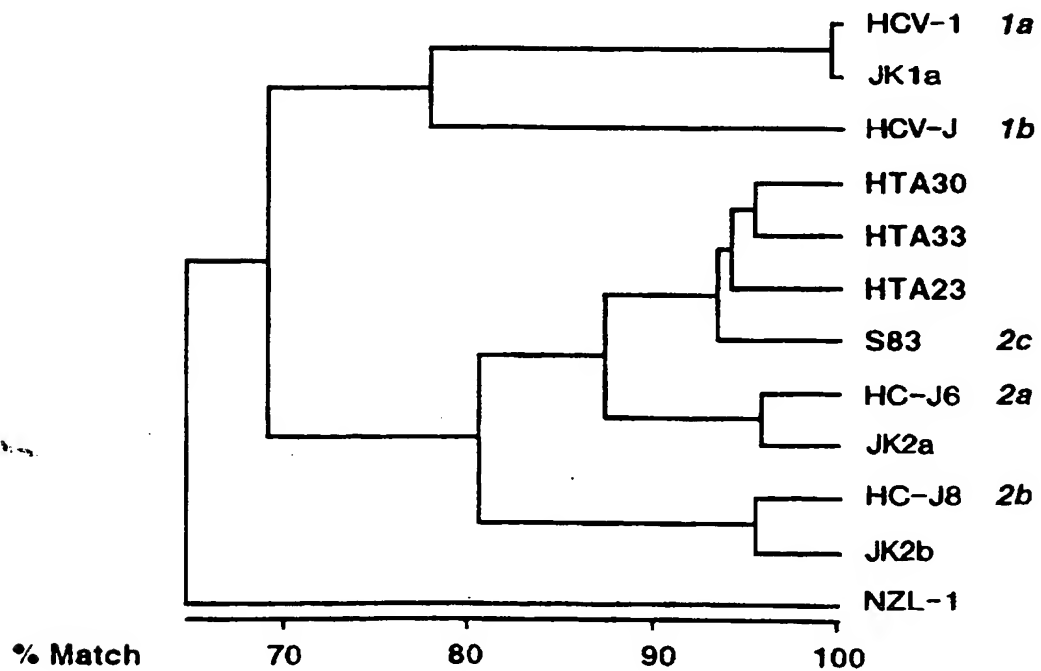


FIG. 2B

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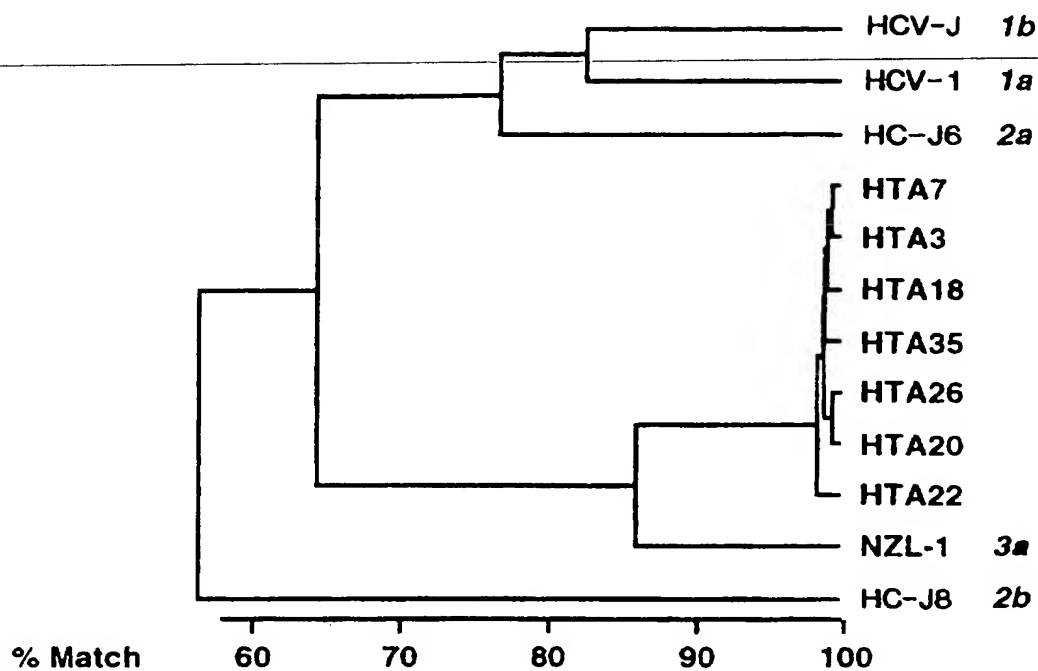


FIG. 2C

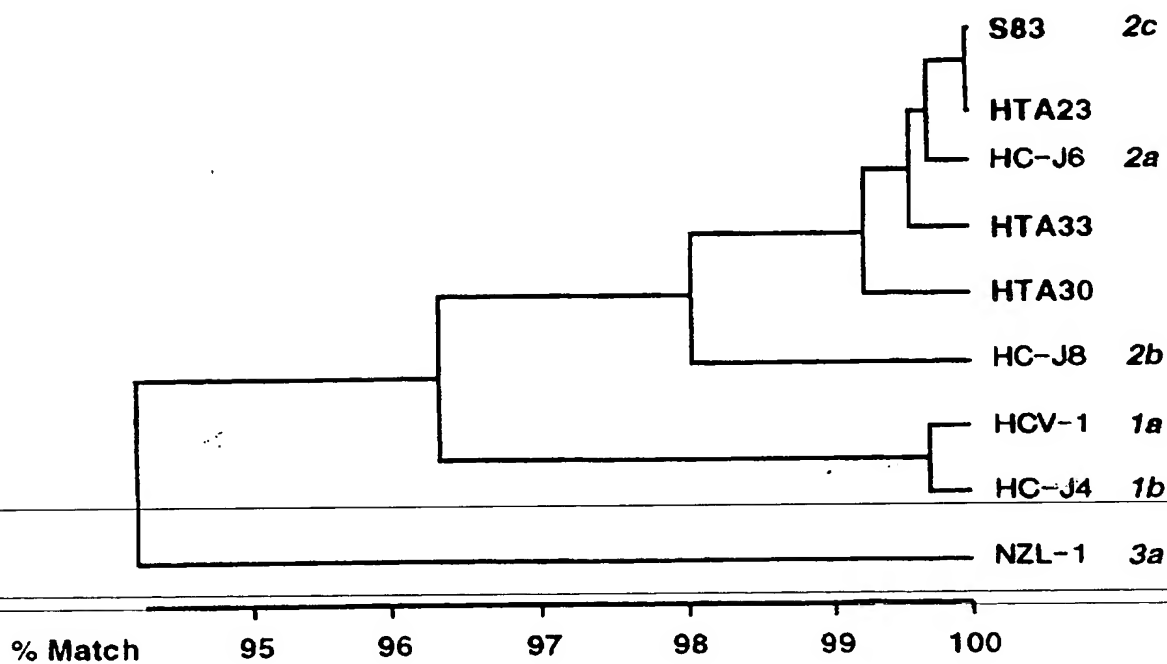


FIG. 2D

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HTA1 AACTCAAGCATTGTGTATGAAGCGGCGGACATGATCATGCACACCCCGGGTGCCTGCCA  
HTA2 AACTCAAGCATCGTGTATGAGGCAGCGGAAGTGATCATGCACATTCCCGGGTGCCTGCCC  
HTA4 AACTCAAGCATAGTATATGAGGCAGCGGACATAATCATGCATACCCCGGGTGCCTGCCC  
HTA24 AACACGAGCATTGTGTATGAGGCAGCGGACTTGATCATGCACGTCCCGGGTGCCTGCCC  
HTA28 AACTCAAGCATCGTGTATGAGGCAGCGGAAGTGATCATGCACATTCTGGGTGCCTGCCC  
HCV-1 AACTCGAGTATTGTGTACGAGGCGGCCGATGCCATCCTGCACACTCCCGGGTGCCTCCCT  
HCV-J AACTCAAGTATTGTGTATGAGGCAGCGGACATGATCATGCACACCCCGGGTGCCTGCCC  
HC-J6 AATGATAGCATTACCTGGCAACTCCAGGCTGCTGTCTCCACGTCCCGGGTGCCTCCCG  
HC-J8 AACAAACGATCACCTGGCAGCTCACTGACGCAGTTCTCCATCTTCTGGATGCGTCCCA  
NZL-1 AATAGCAGTATTGTGTATGAGGCCGATGATGTCTATTCTGCACACACCCGGCTGTGTACCT

HTA1 TCGCTCCGGGAGGGCAATCTCTCCCGCTGCTGGGTAGCGCTCACTCCCACGCTCGCGGCC  
HTA2 TCGCTTCGGGAGAGCAATCTCTCCCGCTGCTGGGTAGCGCTCACCCCACACTCGCGGCC  
HTA4 TGTGTTTCGGGAGGTCAACTCTCCCGCTGCTGGGCAGCGCTCACCCCACACTCGCGGCC  
HTA24 TCGCTTCGGGAGGGCAACTCTCTCCCGATGCTGGGTAGCGCTCACTCCCACGATCGCGGCC  
HTA28 TCGCTTCGGGAGGGCGACTTCTCCCGCTGCTGGGTAGCGCTCACCCCACACTCGCGGCC  
HCV-1 TCGCTTCGTGAGGGCAACGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACC  
HCV-J TCGCTCCGGGAGAGTAATTTCTCCCGTTGCTGGGTAGCGCTCACTCCCACGCTCGCGGCC  
HC-J6 TGCGAGAAAGTGGGGAATACATCTCGGTGCTGGATACCGGTCTCACCGAATGTGGCCGTG  
HC-J8 TGTGAGAATGATAATGGCACCTTGCAATTGCTGGATACAAGTAACACCCAACGTGGCTGTG  
NZL-1 TGTGTCCAGGACGGCAATACATCTACGTGCTGGACCCAGTGACACCTACAGTGGCAGTC  
JK1a TGTGTTTCGCGAGGGCAACGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACC

HTA1 AGAAACAGCAGCGTTCCTACTACGACAATACGACGCCATGTCGACTTGCTAGTAGGAGCG  
HTA2 AGGAACAGCAGCGTCCCCACCACGACAATACGACGCCACGTGCGACTTGCTCGTTGGGGCG  
HTA4 AGGAACCTCCAGCGTGCCCACTACGACAATACGACGCCACGTGCGACTTGCTCGTTGGGGCG  
HTA24 AGGAACAGCAGTGTCCCCGTACGACCAATACGACGCCACGTGCGATTGCTCGTTGGGGCG  
HTA28 AGGAATAACAGCGTCCCCACTACGACAATACGACGCCACGTGCGACTTGCTCGTTGGGGCG  
HCV-1 AGGGATGGCAAACCTCCCCGCGACGCGCTTCGACGTCAATCGATCTGCTTGTGCGGAGC  
HCV-J AGGAACAGCAGCATCCCCACCACGACAATACGACGCCACGTGCGATTGCTCGTTGGGGCG  
HC-J6 CAGCAGCCCGGCGCCCTCACGCAGGGCTTACGACGCACATTTGACATGGTTGTGATGTCC  
HC-J8 AAACACCGCGGTGCGCTCACTCGTAGCCTGCGAACACACGTGCGACATGATCGTAATGGCA  
NZL-1 AGGTACGTGCGAGCAACTACTGCTTCGATACGCAGTCATGTGGACCTATTAGTAGGCGCG

HTA1 GCTGCTTTTTGCTCCGCCATGTACGTGGGGGACCTCTGCGGATCTATTTTCCTCGTCTCC  
HTA2 GCTGCCTTCTGCTCCGCTATGTATGTGGGGGATCTCTGCGGATCTGTTTTCTTGTCTCC  
HTA4 GCTGCTTTCTGCTCCGCTATGTACGTGGGGGATCTATGCGGATCTGTTCTACTTGTCTCT  
HTA24 GCTGCTCTTTGCTCCGCCATGTACGTGGGGGATCTCTGCGGATCTGTCTTCTCGCTTCC  
HTA28 GCTGCCTTCTGCTCCGCTATGTACGTGGGGGATCTCTGCGGATCTGTTTTCTTGTCTCC  
HCV-1 GCCACCCCTCTGTTCCGCCCTCTACGTGGGGGACCTATGCGGGTCTGTCTTTCTTGTCCGC  
HCV-J GCTGCTCTCTGTTCCGCTATGTACGTGGGGGATCTCTGCGGATCCGTTTTCTCGTCTCC  
HC-J6 GCCACGCTCTGCTCCGCTCTTTACGTGGGGGACCTCTGCGGTGGGGTGATGCTTGCAGCC  
HC-J8 GCTACGGCCTGCTCGGCCTTGTATGTGGGAGATGTGTGCGGGGCCGTGATGATTCTATCG  
NZL-1 GCCACGATGTGCTCTGCGCTCTACGTGGGTGATATGTGTGGGGCTGTCTTTCTCGTGGGA

HTA1 CAACTGTTTACCTTCTCGCCCCGCCGGCATCATACAGTACAGGACTGCAATTGCTCGATC  
HTA2 CAACTGTTTACCTTTTTCGCCTCGCCGGCATGAGACAGTACAGGACTGCAATTGTTCAATC  
HTA4 CAGCTGTTTACCTTCTCACCTCGCCGGCACGAGACAGTGCAGGACTGCAATTGTTCAATC  
HTA24 CAGTTGTTCACTTTCTCGCCTCGCCAGCATCAGACGGTACAGGACTGCAACTGCTCAATC  
HTA28 CAACTGTTTACCTTTTTCGCCTCGCCGGCATGCGACAGTACAGGACTGCAATTGTTCAATC

FIG. 3A-1

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HCV-1	CAACTGTTACACCTTCTCTCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATC
HCV-J	CAGCTGTTACACCTTCTCACCTCGCCGGTATGAGACGGTACAAGATTGCAATTGCTCAATC
HC-J6	CAGATGTTCAATTGTCTCGCCACAGCACCCTGGTTTGTGCAAGACTGCAATTGCTCCATC
HC-J8	CAGGCTTTCATGGTATCACCACAACGCCACAACCTTCACCCAAGAGTGCAACTGTTCCATC
NZL-1	CAAGCCTTCACGTTTACAGACCTCGACGCCATCAAACGGTCCAGACCTGTAAGTCTCGCTG

HTA1	TATCCC
HTA2	TATCCC
HTA4	TATCCC
HTA24	TATCCC
HTA28	TATCCC
HCV-1	TATCCC
HCV-J	TATCCC
HC-J6	TACCCT
HC-J8	TACCAA
NZL-1	TACCCA

FIG. 3A-2

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JK1a CGCAACTCCACGGGGCTTTACCACGTACCAATGATTGCCCTAACTCGAGTATTGTGTAC  
JK2a AAGAACACCAGCGACAGCTACATGGTGACCAATGACTGCCAAAATGACAGCATCACCTGG  
JK2b AGGAACATCAGTTCTAGCTACTACGCCACTAATGACTGCTCGAACAACAGCATCACCTGG  
SW83.2c AAGGACACCGGGCGACTCCTACATGCCGACCAACGATTGCTCCAATCTAGTATCGTTTGG  
HTA23 AAAAAACACCAGCATCTCCTATATGGCGACCAACGACTGCTCCAATTCAGCATCGTTTGG  
HTA33 AAGAACACCAGCGACTCCTACATGGCGACTAACGACTGCTCTAACTCCAGCATCGTTTGG  
HTA30 AAGAACACCAGCACCTCCTACATGGTGACTAACGATTGCTCCAATCCAGCATCGTTTGG  
HCV-1 CGCAACTCCACGGGGCTTTACCACGTACCAATGATTGCCCTAACTCGAGTATTGTGTAC  
HCV-J CGCAACGTGTCCGGGATATACCATGTACGAACGACTGCTCCAATCAAGTATTGTGTAT  
HC-J8 AGGAACATTAGTTCTAGCTACTACGCCACTAATGATTGCTCAAAACAACAGCATCACCTGG  
NZL-1 CGGAATACGTCTGGCCTCTACGTCTTACCAACGACTGTTCCAATAGCAGTATTGTGTAT  
HC-J6 AAGAACATCAGTACCGGCTACATGGTGACCAACGACTGCACCAATGATAGCATTACCTGG  
\* \*\* \* \* \* \*

JK1a GAGACGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGTGTTTCGCGAGGGCAAC  
JK2a CAGCTTGAGGCTGCGGTCTCCACGTCCCGGGTGCGTCCCGTGCGAGAGTGGGAAAT  
JK2b CAGCTCACCAACGCAGTTCTCCACCTTCCCGGATGCGTCCCATGTGAGAATAATAATGGC  
SW83.2c CAGCTTGAAGGAGCAGTGCTTCATACTCCTGGATGCGTCCCTTGTGAGCGTACCGCCAAC  
HTA23 CAGTTTGACGGCGCAGTGCTCCATACTCCTGGATGTGTCCCTTGCGAACGGACCGGCAAC  
HTA33 CAGCTTGAGGACGCAGTGCTCCATGTCCCTGGATGTGTCCCTTGTGAGAAGACTGGCAAT  
HTA30 CAACTTGAAGGCGCAGTGCTCCATGTTCCTGGATGTGTCCCTTGTGAGCAGATCGGCAAC  
HCV-1 GAGGCGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCCTTGTGAGGGCAAC  
HCV-J GAGGACGCGGACATGATCATGCACACCCCGGGTGCGTGCCCTGCGTCCGGGAGAGTAAT  
HC-J8 CAGCTCACTGACGCAGTTCTCCATCTTCTGGATGCGTCCCATGTGAGAATGATAATGGC  
NZL-1 GAGGCGGATGATGTCACTTCTGCACACCCCGGCTGTGTACCTTGTGTCCAGGACGGCAAT  
HC-J6 CAACTCCAGGCTGCTGTCTCCACGTCCCGGGTGCGTCCCGTGCGAGAAAGTGGGGAAT  
\* \* \* \* \*

JK1a GCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACCTCCCC  
JK2a ACATCTCGGTGCTGGATACCGGTCTCACCAACCGTGGCTGTGCGGCAGCCGGCGCCCTC  
JK2b ACCTTGCAATTGCTGGATACAAGTAACACCTAATGTGGCCGTAAACATCGCGGCGCACTC  
SW83.2c GTCTCTCGATGTTGGGTGCCGGTTGCCCCCAATCTCGCCATAAGTCAACCTGGCGCTCTC  
HTA23 GCGTCCCGGTGTTGGGTGCCGGTTGCCCCCAATGTGGCTATAAGACAACCCGGCGCCCTC  
HTA33 ACGTCTCGGTGCTGGGTGCCGGTTACCCCAATGTGGCTACAAGTCAACCCGGCGCTCTC  
HTA30 GTGCTCAGTGTTGGGTGCCGGTTACCCCAATATGGCCATAAGTACACCCGGCGCTCTC  
HCV-1 GCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGATGGCAAACCTCCCC  
HCV-J TTCTCCCGTTGCTGGGTAGCGCTCACTCCCACGCTCGCGGCCAGGAACAGCAGCATCCCC  
HC-J8 ACCTTGCAATTGCTGGATACAAGTAACACCCCAACGTGGCTGTGAAACACCGGGTGGCTC  
NZL-1 ACATCTACGTGCTGGACCCAGTGACACCTACAGTGGCAGTCAGGTACGTCCGAGCAACT  
HC-J6 ACATCTCGGTGCTGGATACCGGTCTCACCGAATGTGGCCGTGCAGCAGCCGGCGCCCTC  
\* \* \* \* \*

JK1a GCGACGCAGCTTCGACGTACATCGATCTGCTTGTGCGGAGCGCCACCCTCTGTTCGGCC  
JK2a ACGCAGGGCTTGCGGACGCACATCGACATGATTGTGATGTCCGCCACGCTCTGCTCCGCT  
JK2b ACTCACAACCTGCGGACACATGTGACATGATCGTAATGGCAGCTACGGTCTGTTCTGGCC  
SW83.2c ACTAAGGGCCTGCGAGCACACATCGATATCATCGTGATGTCTGCTACGGTCTGTTCTGCC  
HTA23 ACTAAGGGCATACGAACGCACATTGATGTCATCGTAATGTCTGCTACGCTCTGTTCTGCC  
HTA33 ACCAGGGGCTTGCGGACGCACATCGATGTCATCGTGATGTACGCCACGCTCTGCTCCGCT  
HTA30 ACTAAGGGCTTGCGAACGCACATCGACGGCATCGTGATGTCCGCTACGCTCTGTTCTGCC  
HCV-1 GCGACGCAGCTTCGACGTACATCGATCTGCTTGTGCGGAGCGCCACCCTCTGTTCTGCC  
HCV-J ACCACGACAATACGACGCCACGTGATTTGCTCGTTGGGGCGGCTGCTCTCTGTTCCGCT  
HC-J8 ACTCGTAGCCTGCGAACACACGTGACATGATCGTAATGGCAGCTACGGCCTGCTCGGCC  
NZL-1 ACTGCTTCGATACGCAGTCATGTGGACCTATTAGTAGGCGCGGCCACGATGTGCTCTGCC  
HC-J6 ACGCAGGGCTTACGGACGCACATTGACATGGTTGTGATGTCCGCCACGCTCTGCTCCGCT  
\* \* \* \* \*

JK1a CTCTACGTGGGGGATCTGTGCGGGTCTGTCTTTCTTGTGCGCCAACCTGTTTACCTTCTCT

FIG. 3B-1

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JK2a	CTCTACGTGGGGGACCTCTGTGGCGGGATGATGCTCGCAGCCCAGATGTTTCATCGTTTCG
JK2b	TTGTACGTAGGAGACGTGTGTGGGGCTGTGATGATTGTGTCTCAGGCCCTTATAATATCA
SW83.2c	CTTTATGTGGGGGACGTGTGTGGCGCGCTGATGCTGGCCGCTCAGGTCGTTCGTGTCTCG
HTA23	CTTTACGTGGGGGACGTGTGTGGTGGCGCTGATGATTGCCGCTCAGGTCGTTCATTGTGTCT
HTA33	CTCTATGTGGGGGACGTGTGTGGCGCGTTGACGATAGCCGCTCAGGTTGTTCATCGTATCG
HTA30	CTTTATGTGGGGGACGTGTGTGGCGCGTTGATGATAGCCGCCAGGTCGTTCATCGTATCG
HCV-1	CTCTACGTGGGGGACCTATGCGGGTCTGTCTTTCTTGTGGGCCAACTGTTACCTTCTCT
HCV-J	ATGTACGTTGGGGATCTCTGCGGATCCGTTTTTCTCGTCTCCCAGCTGTTACCTTCTCA
HC-J8	TTGTATGTGGGAGATGTGTGCGGGGCGTGATGATTCTATCGCAGGCTTTCATGGTATCA
NZL-1	CTCTACGTGGGTGATATGTGTGGGGCTGTCTTTCTCGTGGGACAAGCCTTCACGTTTCTCA
HC-J6	CTTTACGTGGGGGACCTCTGCGGTGGGGTGATGCTTGCAGCCCAGATGTTTCATTGTCTCG
	* * * * *
JK1a	CCCAGGCGCCACTGGACGACGCAAGGTTGCAAT
JK2a	CCGCAGAACCCTGGTTTCGTGCAGGAATGCAAT
JK2b	CCAGAACACCATAACTTCACCCAAGAGTGCAAC
SW83.2c	CCACAACACCATACTGTTTGTCCAGGAATGCAAC
HTA23	CCGCAGCATCACCCTTTGTCCAGGACTGCAAT
HTA33	CCACGGCACCACCCTTTGTCCAGGACTGCAAT
HTA30	CCACAGCACCACCCTTTGTCCACGACTGCAAC
HCV-1	CCCAGGCGCCACTGGACGACGCAAGGTTGCAAT
HCV-J	CCTCGCCGGTATGAGACGGTACAAGATTGCAAT
HC-J8	CCACAACGCCACAACCTTCACCCAAGAGTGCAAC
NZL-1	CCTCGACGCCATCAAACGGTCCAGACCTGTAAC
HC-J6	CCACAGCACCCTGGTTTGTGCAAGACTGCAAT
	** * **

FIG. 3B-2

HTA3	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCCTTACCAACGA
HTA7	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCCTTACCAACGA
HTA18	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCCTTACCAACGA
HTA20	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCCTTACCAACGA
HTA22	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCCTTACCAACGA
HTA26	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCCTTACCAACGA
HTA35	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCCTTACCAACGA
NZL-1	TCATCCAGCAGCCAGTCTAGAGTGGCGGAATACGTCTGGCCTCTACGTCCTTACCAACGA
HCV-1	TGTGCCCCGCTTCGGCCTACCAAGTGCGCAACTCCACGGGGCTTTACCACGTCACCAATGA
HCV-J	CATCCCAGCTTCCGCTTACGAGGTGCGCAACGTGTCCGGGATATACCATGTCACGAACGA
HC-J6	CACCCCGGTCTCCGCTGCCGAAGTGAAGAACATCAGTACCGGCTACATGGTGACCAACGA
HC-J8	AGTGCCAGTGTCTGTCAGTGGGAAGTCAGGAACATTAGTTCTAGCTACACGGGCTATGTA

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HTA3 CTGTTCCAATAACATTATTGTGTATGAGGCCGATGACGTCATCCTGCACACGCCCGGCTG  
HTA7 CTGTTCCAATAACATCATTGTGTATGAGGCCGATGACGTCATCCTGCACGACCCGGCTG  
HTA18 CTGTTCCAATAATATTATTGTGTATGAGGCCGACGACGTCATCCTGCACGCCCCGGCTG  
HTA20 CTGTTCCAATAACATTATTGTGTATGAGGCCGATGACGTCATCCTGCACACACCCGGCTG  
HTA22 CTGTTCCAATAACAGTATTGTGTATGAGGCCGATCACGTCATCCTGCACACACCCGGCTG  
HTA26 CTGTTCTAATAACATTATTGTGTATGAGGCCGATGACGTCATCCTGCACACACCCGGCTG  
HTA35 CTGTTCCAACAACATTATTGTGTATGAGGCCGATGACGTCATTCTGCACACGCCCGGCTG  
NZL-1 CTGTTCCAATAGCAGTATTGTGTATGAGGCCGATGATGTCATTCTGCACACACCCGGCTG  
HCV-1 TTGCCCTAACTCGAGTATTGTGTACGAGGCCGCCGATGCCATCCTGCACACTCCGGGGTG  
HCV-J CTGCTCCAACCTCAAGTATTGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGGTG  
HC-J6 CTGCACCAATGATAGCATTACCTGGCAACTCCAGGCTGCTGTCTCCACGTCCTCCGGGTG  
HC-J8 TTGCTCAAACAACAGCATCACCTGGCAGCTCACTGACGCGAGTTCTCCATCTTCCTGGATG

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HTA3 TGTACCTTGTGTTT CAGGACGGTAATACATCCAAGTGCTGGACCCCAAGTGACACCTACAGT  
HTA7 TGTACCTTGTGTTT CAGGACGGCAATACATCCACGTGCTGGACCCCAAGTGACACCTACAGT  
HTA18 TGTACCTTGTGTTT CAGGACGGCAATACATCCAAGTGCTGGATCCCAGTGACACCTACAGT  
HTA20 TGTACCTTGTGTTT CAGGACGGCAATACATCCACGTGCTGGACCCCAAGTGACACCTACAGT  
HTA22 TGTACCTTGTGTTT CAGGACGGCAATACATCCACGTGCTGGACCCCAAGTGACACCTACAGT  
HTA26 TGTACCTTGTGTTT CAGGACGGCAATGCATCCACGTGCTGGACCCCAAGTAACACCTACAGT  
HTA35 CGTACCTTGTGTACAGGACGGCAATACATCCAAGTGCTGGACCCCAAGTGACACCTACAGT  
NZL-1 TGTACCTTGTGTCCAGGACGGCAATACATCTACGTGCTGGACCCCAAGTGACACCTACAGT  
HCV-1 CGTCCCTTGCCTTCTGAGGGCAACGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGT  
HCV-J CGTGCCCTGCGTCCGGGAGAGTAATTTCTCCCGTTGCTGGGTAGCGCTCACTCCCAACGCT  
HC-J6 CGTCCCGTGCGAGAAAGTGGGGAATACATCTCGGTGCTGGATACCGGTCTCACC GAATGT  
HC-J8 CGTCCCATGTGAGAATGATAATGGCACCTTGCATTGCTGGATACAAGTAACACCAACGTT  
\* \* \* \* \*

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HTA3 GGCAGTCAGGTACGTCGGAGCAACCACCGCTTCAATACGCAGCCACGTGGACCTATTATT  
HTA7 GGCAGTCAGGTACGTCGGAGCAACCACCGCTTCAATACGCAGCCATGTGGACCTATTAGT  
HTA18 GGCAGTCAGGTACGCCGGAGCAACCACCGCTTCAATACGCAGCCATGTGGACCTGTTAGT  
HTA20 ATCAGTCAGGTACGTCGGAGCAACCACCGCTTCAATACGCAGCCATGTGGACCTACTATT  
HTA22 ATCAGTCGAGTACGTCGGAGCAACCACCGCTTCAATACGCAGCCATGTGGACCTACTATT  
HTA26 ATCAGTCAGGTACGTCGGAGCAACCACCGCTTCAATACGCAGCCATGTGGACCTACTATT  
HTA35 GGCAGTCAGGTACGTCGGAGCAACTACCGCTTCAATACGCAGCCATGTGGACCTATTATT  
NZL-1 GGCAGTCAGGTACGTCGGAGCAACTACTGCTTCGATACGCAGTCATGTGGACCTATTAGT  
HCV-1 GGCCACCAGGGATGGCAAACCTCCCCGCGACGCAGCTTCGACGTCACATCGATCTGCTTGT  
HCV-J CGCGGCCAGGAACAGCAGCATCCCCACCACGACAATACGACGCCACGTCGATTGCTCGT  
HC-J6 GGCCGTGCAGCAGCCCGGCGCCCTCACGCAGGGCTTACGGACGCACATTGACATGGTTGT  
HC-J8 GGCTGTGAAACACCGCGGTGCGCTCACTCGTAGCCTGCCGAACACAGTCGACATGATCGT

**\*                 ★          ★                                 ★                                 ★      ★★                 ★★         ★   ★★         ★   ★         ★**

HTA3 GGGCGCGGCCACGATGTGCTCTGCGCTCTACGTGGGT

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HTA7	GGGCGCGGCCACGATGTGCTCTGCGCTCTACGTGGGT
HTA18	GGGCGCGGCCACGATGTGCTCTGCGCTCTACGTGGGT
HTA20	GGGCGCGGCCACGATGTGCTCCGCGCTCTACGTGGGT
HTA22	GGGCGCGGCCACGATGTGCTCTGCGCTCTACGTGGGT
HTA26	GGGCGCGGCCACGATGTGCTCTGCGCTCTATGTGGGT
HTA35	GGGCGCGGCCACGATGTGCTCTGCGCTCTACGTGGGT
NZL-1	AGGCGCGGCCACGATGTGCTCTGCGCTCTACGTGGGT
HCV-1	CGGGAGCGCCACCCTCTGTTCCGCCCTCTACGTGGGG
HCV-J	TGGGGCGGCTGCTCTCTGTTCCGCTATGTACGTTGGG
HC-J6	GATGTCCGCCACGCTCTGCTCCGCTCTTTACGTGGGG
HC-J8	AATGGCAGCTACGGCCTGCTCGGCCTTGTATGTGGGA

\* \* \* \* \*

FIG. 3C-2

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HC-J6 -GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTACAGCCTCCAGGCCCCCCCC  
HC-J8 -GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTACAGCCTCCAGGCCCCCCCC  
HC-J4 -GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCCC  
HCV-1 -GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCCC  
NZL1 -GCGGAAAGCGCCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGACCCCCCCCC  
S83 -GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTACAGCCTCCAGGCCCCCCCC  
HTA23 -GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTACAGCCTCCAGGCCCCCCCC  
HTA30 -GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTACAGCCTCCAGGCCCCCCCC  
HTA33 ~~GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTACAGCCTCCAGGCCCCCCCC~~  
\*\*\*\*\*

HC-J6 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAGAC  
HC-J8 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAGAC  
HC-J4 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGAC  
HCV-1 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGAC  
NZL1 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATCGCTGGGGTGAC  
S83 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAGAC  
HTA23 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAGAC  
HTA30 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGAAGAC  
HTA33 TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAGAC  
\*\*\*\*\*

HC-J6 TGGGTCCTTTCTTGGATAAAACCCACTCTATGCCCGGTCATTTGGGCGTGCCCCGCAAGA  
HC-J8 TGGGTCCTTTCTTGGATAAAACCCACTCTATGCCCGGTCATTTGGGCGTGCCCCGCAAGA  
HC-J4 CGGGTCCTTTCTTGGATCAACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCGCGAGA  
HCV-1 CGGGTCCTTTCTTGGATCAACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCGCAAGA  
NZL1 CGGGTCCTTTCTTGGAGCAACCCGCTCAATACCCAGAAATTTGGGCGTGCCCCGCGAGA  
S83 TGGGTCCTTTCTTGGATAAAACCCACTCTATGCCCGGCCATTTGGGCGTGCCCCGCAAGA  
HTA23 TGGGTCCTTTCTTGGATAAAACCCACTCTATGCCCGGCCATTTGGGCGTGCCCCGCAAGA  
HTA30 TGGGTCCTTTCTTGGATAAAACCCACTCTATGCCCGGCCATTTGGGCGTGCCCCGCAAGA  
HTA33 TGGGTCCTTTCTTGGATAAAACCCACTCTATGCCCGGCCATTTGGGCGTGCCCCGCAAGA  
\*\*\*\*\*

HC-J6 CTGCTAGCCGAGTAG  
HC-J8 CTGCTAGCCGAGTAG  
HC-J4 CTGCTAGCCGAGTAG  
HCV-1 CTGCTAGCCGAGTAG  
NZL1 TCACTAGCCGAGTAG  
S83 CTGCTAGCCGAGTAG  
HTA23 CTGCTAGCCGAGTAG  
HTA30 CTGCTAGCCGAGTAG  
HTA33 CTGCTAGCCGAGTAG  
\*\*\*\*\*

FIG. 3D

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/06062

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12Q1/68 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	HEPATOLOGY, vol. 20, no. 4, - October 1994 page 244a XP002035908 MURASHIMA S. ET AL.,: "Analysis of HCV genome population by PCR heteroduplex method" see the whole document --- -/-	12-23

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

24 July 1997

Date of mailing of the international search report

07.08.97

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Authorized officer

Müller, F

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International Application No

PCT/US 97/06062

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X	WO 92 19743 A (CHIRON CORP) 12 November 1992 see page 125, line 10 ---	2
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